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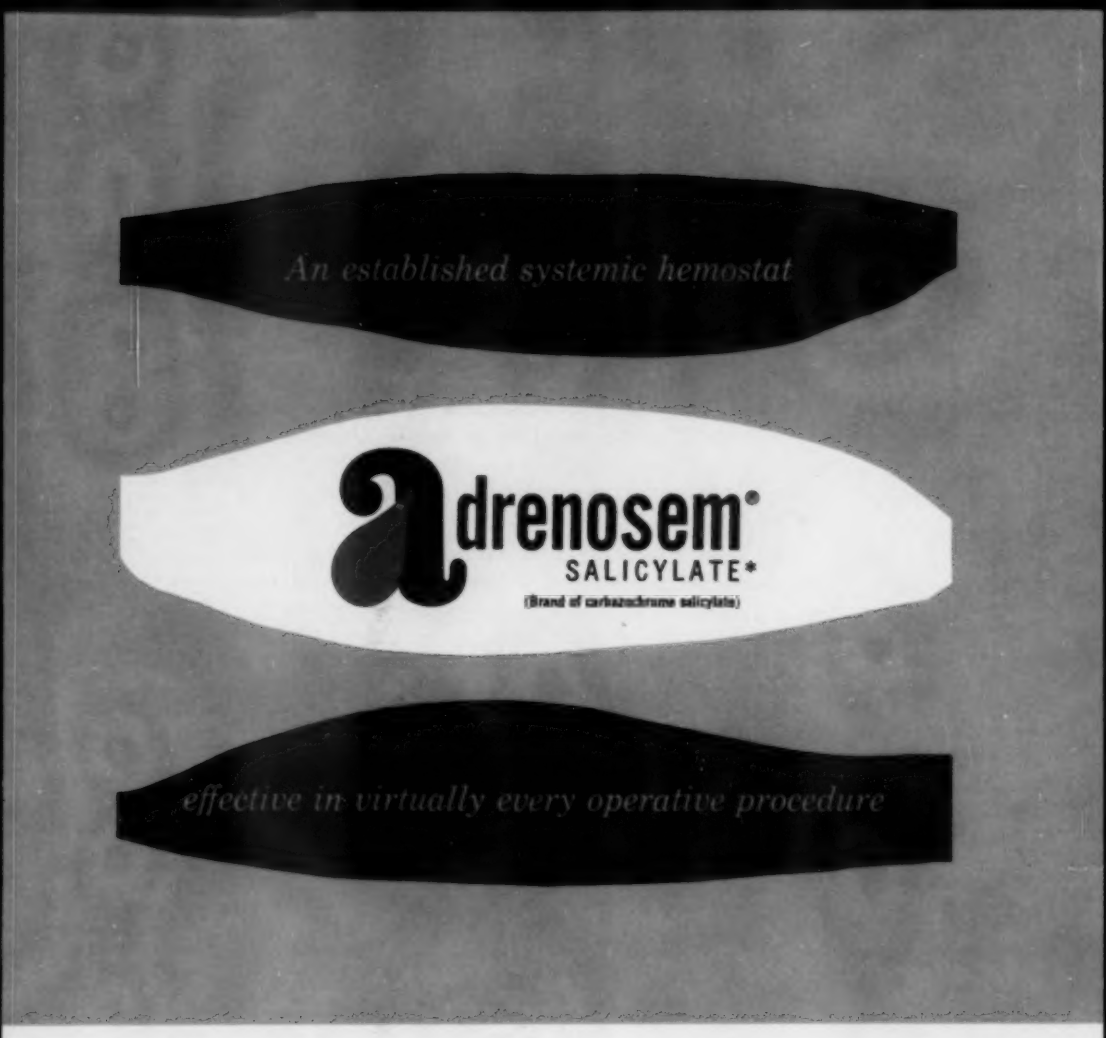

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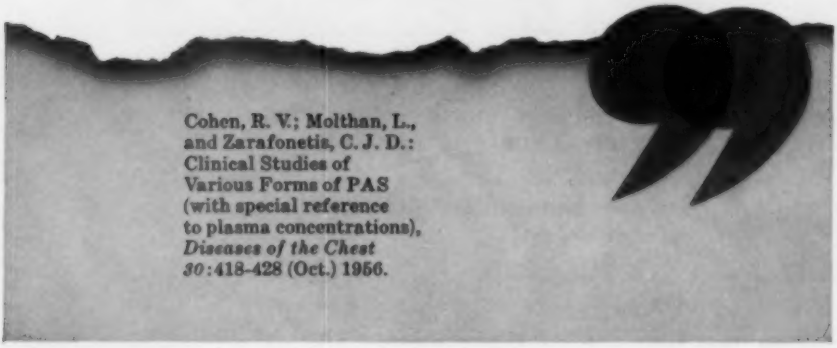
Cohen, R. V.; Molthan, L., and Zarafonetis, C. J. D.: Clinical Studies of Various Forms of PAS (with special reference to plasma concentrations), *Diseases of the Chest* 30:418-428 (Oct.) 1956.

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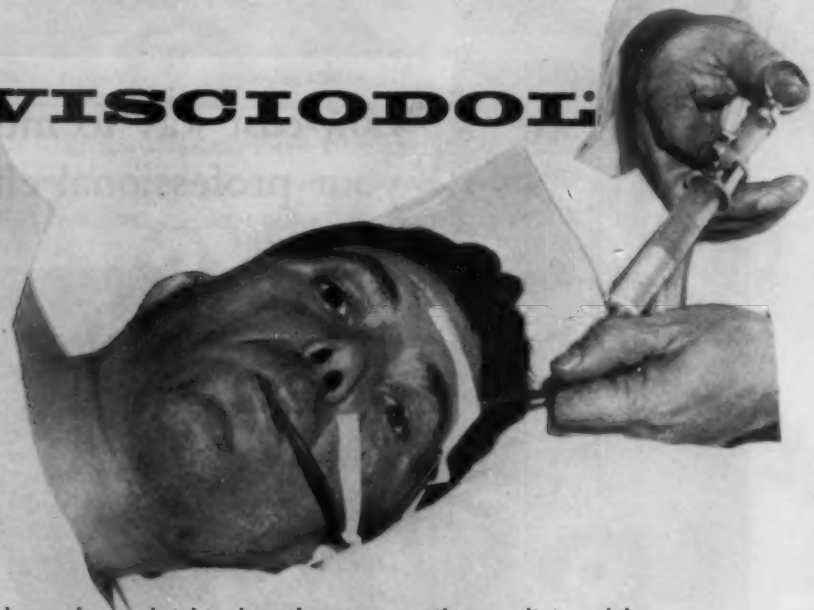

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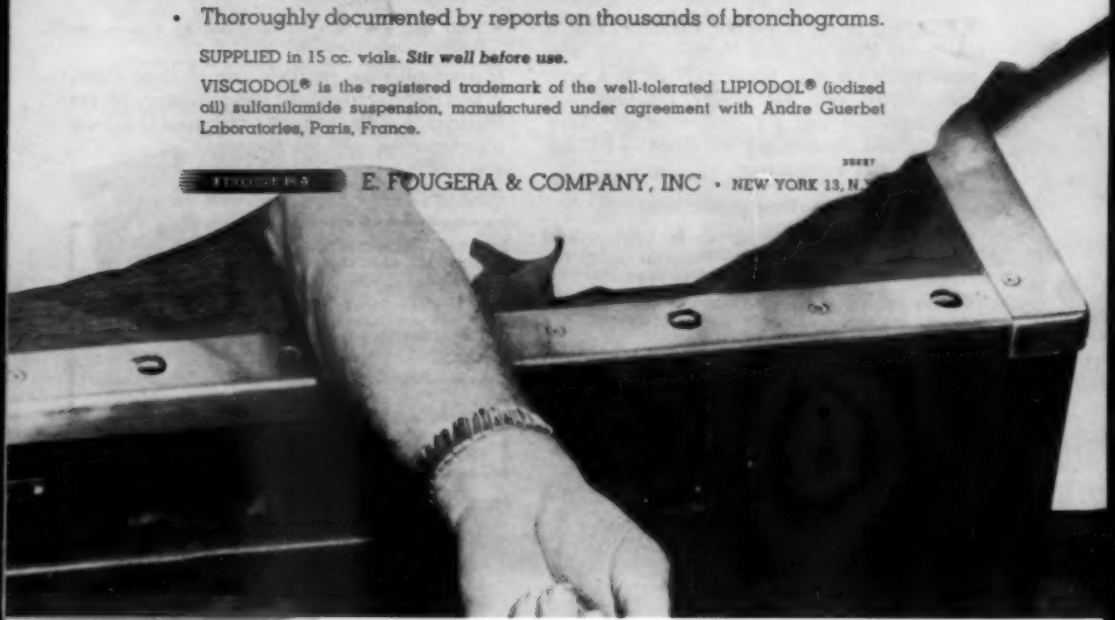

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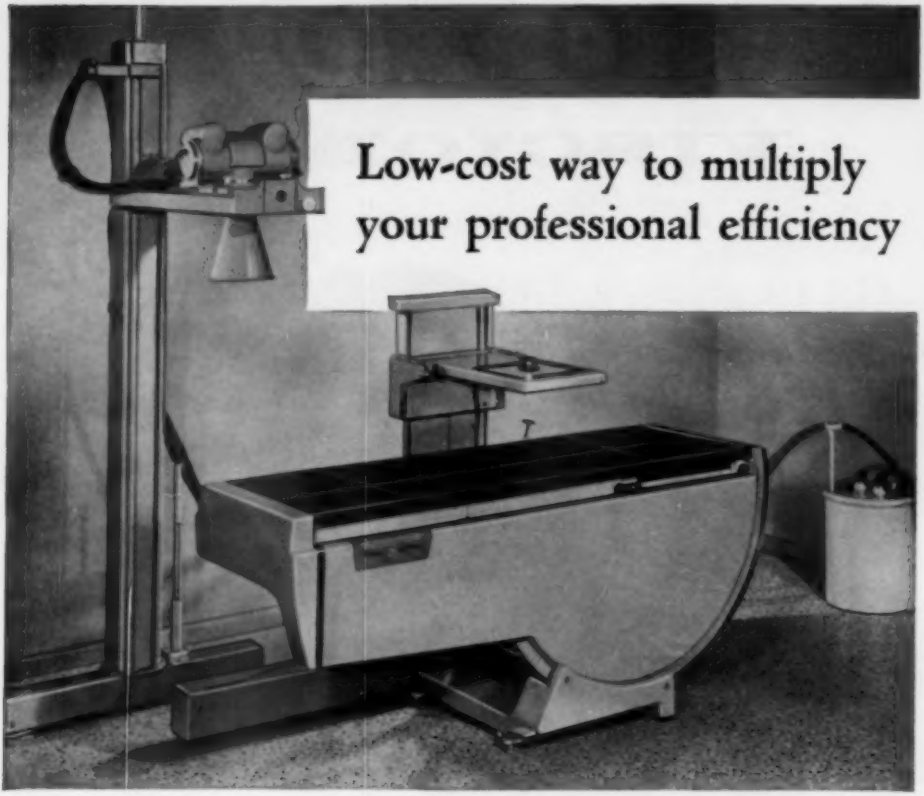

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THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES

VOLUME 76 *September, 1957* NUMBER 3

CHEMOTHERAPY OF TUBERCULOSIS[1]

I. Antituberculous Activity of Verazide and Related Hydrazones

SYDNEY D. RUBBO, JANICE EDGAR, AND GEOFFREY VAUGHAN

(Received for publication May 13, 1957)

Introduction

In a preliminary publication (1), the *in vivo* activity of Verazide, 1-*iso*-nicotinoyl-2-veratrylidene hydrazine or 3:4-dimethoxybenzal *iso*nicotinyl hydrazone, was described. It was shown that Verazide exerted a marked antituberculous activity in experimental guinea pig tuberculosis and that the acute toxicity in mice was about one-third that of isoniazid. The present paper deals with a more detailed report on Verazide and related hydrazones. Some of these hydrazones can be claimed to possess a higher therapeutic index than isoniazid in experimental tuberculosis, since they combine a high isoniazid type of activity with an extremely low toxicity. This fact has been recognized by other authors (2–6) and two hydrazones, 3-methoxy-4-hydroxybenzal (Phtivazid® in USSR) and 1-hydroxybenzal (Salizid® in the United States or Nupasal® 213 in England) have been used in the treatment of pulmonary tuberculosis.

Materials and Methods

Bacteriology

In vitro tests: The technique used in this laboratory for determining tuberculostatic activity has been described previously (7) and has been used in the present studies. The inoculum, approximately 10^6 viable units, was prepared from well-dispersed cells of *M. tuberculosis* H37Rv grown in Dubos-Davis Tween®-albumin medium. The test medium containing drug dilutions was a modified Proskauer and Beck medium described here as synthetic medium; when enriched with 10 per cent ox serum it is referred to as serum synthetic medium. The tuberculostatic end point was taken as the highest dilution inhibiting visible growth after fourteen days' incubation at 37°C.

In vivo tests: The healing ulcer method, previously described by one of the present writers (8), was followed. A highly virulent human strain of *M. tuberculosis* Petty was used in all *in vivo* tests.

Preparation of resistant strains: The rate at which resistance to isoniazid or Verazide developed was determined by using for each progressive tuberculostatic titration an inoculum from the tube containing the highest concentration of drug in which growth occurred. Resistant strains so isolated were examined for their stability and degree of resistance and cross-resistance.

[1] From the School of Bacteriology, University of Melbourne, Carlton, Victoria, Australia.

Chemistry

The Schiff's bases of isonicotinic acid hydrazide were prepared by refluxing isoniazid (1 mole) with the required aldehyde (1 mole) in absolute alcohol for one hour. On allowing the solution to cool, the hydrazone crystallized out. It was then filtered off and subjected to further recrystallization from absolute alcohol to give a 75 to 85 per cent yield of pure product.

The metal complexes listed were prepared by mixing a solution of the required A.R. metal salt (1 mole) in aqueous methanol (50 per cent) with a solution of isonicotinic acid hydrazide, or the Schiff's base (1 mole), in methanol. The metal complex was obtained as a crystalline precipitate, collected by filtration and washed with methanol and finally with water.

All the compounds listed in figure 1 were dried over phosphorus pentoxide at 100°C. at 1 mm. of mercury for one hour unless otherwise mentioned. The names in parentheses given after the chemical name are, with the exception of Verazide and Salizid, unofficial laboratory designations used merely for convenience.

3:4-Dimethoxybenzal isonicotinyl hydrazone (*Verazide*) *I:* From isoniazid and veratric aldehyde. Small, almost colorless needles, m.p. 188°C. Found: C, 63.2; H, 5.1. $C_{15}H_{15}N_3O_3$ required C, 63.2; H, 5.3 per cent.

3-Methoxy 4-hydroxybenzal isonicotinyl hydrazone (*vanizide*) *II:* From isoniazid and vanillin. Bright yellow needles, m.p. 229°C. Found: C, 61.8; H, 5.0. $C_{14}H_{13}N_3O_3$ requires C, 62.0; H, 4.8 per cent.

2-Hydroxy 3-methoxybenzal isonicotinyl hydrazone (*orthovanizide*) *III:* From isoniazid and

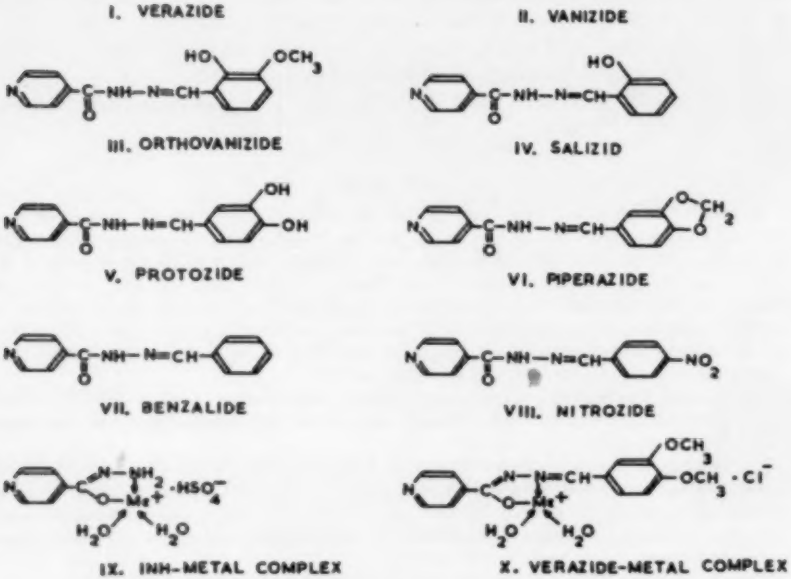


FIG. 1. Formulae of compounds used in the present study (*see* text).

o-vanillin. Pale yellow needles, m.p. 230°C. Found: C, 62.1; H, 4.8. $C_{14}H_{12}N_2O_3$ requires C, 62.0; H, 4.8 per cent.

2-Hydrozybenzal isonicotinyl hydrazone (Salizid) IV: From isoniazid and salicylaldehyde. Almost colorless plates, m.p. 249°C. Found: C, 65.0; H, 4.8. $C_{12}H_{11}N_3O_2$ requires C, 64.7; H, 4.6 per cent.

3-,4-Dihydroxybenzal isonicotinyl hydrazone (protozide) V: From isoniazid and protocatechuic aldehyde. Yellow-brown needles, m.p. 225°C. (d). Found: C, 57.1; H, 4.9. $C_{13}H_{11}N_3O_3 \cdot H_2O$ requires C, 56.8; H, 4.8 per cent.

3,4-Methylenedioxybenzal isonicotinyl hydrazone (piperazide) VI: From isoniazid and piperonal. White needles, m.p. 230°C. Found: C, 62.7; H, 4.2. $C_{14}H_{11}N_3O_3$ requires C, 62.5; H, 4.1 per cent.

Benzal isonicotinyl hydrazone (benzalide) VII: From isoniazid and benzaldehyde. White needles, m.p. 198°C. Found: C, 69.5; H, 4.9. $C_{13}H_{11}N_3O$ requires C, 69.3; H, 4.9 per cent.

4-Nitrobenzal isonicotinyl hydrazone (nitrozide) VIII: From isoniazid and 4-nitrobenzaldehyde. White needles, m.p. 274°C. Found: C, 58.1; H, 4.0. $C_{13}H_{10}N_4O_3$ requires C, 57.8; H, 3.7 per cent.

Isoniazid-ferrous sulfate complex IX: Brown powder, m.p. >360°C., air dried Analysis to be published elsewhere.

Isoniazid-cobalt sulphate complex IX: Pink powder, m.p. >360°C., air dried. Analysis to be published elsewhere.

Isoniazid-zinc sulfate complex IX: White powder, m.p. >360°C., air dried. Analysis to be published elsewhere.

Isoniazid-copper sulfate complex IX: Green powder, m.p. >360°C., air dried. Analysis to be published elsewhere.

Verazide-copper chloride complex X: Green powder, m.p. >360°C., air dried. Analysis to be published elsewhere.

Results

In vitro tuberculostatic activity: The *in vitro* activity of isoniazid, Verazide, and six other closely related hydrazones is recorded in table 1. It may be seen that they all exhibit a high degree of tuberculostatic activity which, in general, is equal to that of the parent substance, isoniazid. Within the group, however, Verazide was consistently slightly more active than the other derivatives.

The influence of serum protein on the tuberculostatic end point was de-

TABLE 1

Tuberculostatic Activity of Hydrazones of Isoniazid

Compound (see figure 1)	Laboratory Name	Highest Dilution Inhibiting Growth of H37Rv in Serum-Synthetic Medium	
		*1M**	*γ per ml.*
I	Verazide	4,096,000	0.07
II	Vanizide (Phtivazid)	2,048,000	0.13
III	Orthovanizide	1,024,000	0.26
IV	Salizid	1,024,000	0.24
V	Protozide	1,024,000	0.25
VI	Piperazide	2,048,000	0.13
VII	Benzalide	2,048,000	0.11
VIII	Nitrozide	1,024,000	0.26
	Isoniazid (for comparison)	2,048,000	0.07

* Reciprocal of the molar concentration.

termined, using isoniazid and Verazide as typical of the group. The end points in the presence or absence of serum were essentially the same for the two drugs.

In an earlier report (9) it was shown that chelation with divalent metals was one essential prerequisite determining the antituberculous activity of isoniazid and Verazide. Although the nature of the metal involved in the drug-parasite system was unknown, it was thought worth while to examine the activity of some metal complexes of isoniazid and Verazide. Such information could provide some indication of the chemotherapeutically active metal complex. Accordingly, the iron, cobalt, zinc, and copper complexes of isoniazid and the copper complex of Verazide were tested for tuberculostatic activity.

The results of these tests are recorded in table 2. In general, the activity of all of the metal complexes was equal to that of the uncomplexed drug. In each case the complex, in contrast to the parent drug, was less active in serum synthetic medium than in the synthetic medium. It did not appear from these results that the role of a potentiator (or antagonizer) of isoniazid activity could be assigned to any of the metals examined. However, the marked antagonistic effect of serum on the activity of the copper complex would suggest that this is not the metal involved in the biologic action of isoniazid and Verazide.

It might be anticipated from the close chemical similarity between isoniazid and its hydrazones that their mode of action is the same. One way of establishing this point would be the demonstration of cross-resistance of isoniazid- and Verazide-resistant strains. That these resistant strains can be isolated readily is shown in table 3. It will be seen that resistant mutants were isolated after prolonged incubation (twenty-three days) in the first titration of each drug. Progressive titrations favoring the selection of resistant mutants soon showed that resistance to isoniazid and Verazide was rapid and of a high order, most probably a one-step mutational pattern. Resistant mutants were also easily derived from Petty and BCG strains, all of which were catalase negative.

The cross-resistance between these mutants is well shown in table 4. There can be little doubt that the mode of action of isoniazid and Verazide is fundamentally the same, and any differences which can be measured chemotherapeutically arise from differences in their pharmacologic, rather than antituberculous, attributes.

TABLE 2

TUBERCULOSTATIC ACTIVITY OF METAL COMPLEXES OF ISONIAZID AND THE COPPER COMPLEX OF VERAZIDE

Complex	Highest Dilution Intramuscularly Inhibiting Growth of H37Rv *IM*	
	Synthetic Medium	Serum-synthetic Medium
Isoniazid-$FeSO_4 \cdot 2H_2O$	8,192,000	2,048,000
Isoniazid-$CoSO_4 \cdot 2H_2O$	4,096,000	2,048,000
Isoniazid-$ZnSO_4 \cdot 2H_2O$	16,384,000	2,048,000
Isoniazid-$CuSO_4 \cdot 2H_2O$	4,096,000	512,000
Verazide-$CuCl_2 \cdot 2H_2O$	8,192,000	1,024,000

TABLE 3

DEVELOPMENT OF DRUG-RESISTANT MUTANTS OF M. TUBERCULOSIS H37Rv

Drug	Titration	Incubation Time	Tuberculostatic End Point (γ/ml.)								Increase in Resistance
			68	34	17	8.5	4.2	2.1	1.5	0.7	
		days									
Isoniazid	First	23	−	−	−	−	−	−	+	+	30 fold
	Second	19	−	+	+	+	+	+	+	+	1,000 fold
	Third	14	−	+	+	+	+	+	+	+	1,000 fold
	Fourth	14	−	+	+	+	+	+	+	+	1,000 fold
			568	284	142	71	36	18	9	4.5	2.2	
Verazide	First	23	−	−	−	−	−	−	−	−	+	60 fold
	Second	19	−	−	−	+	+	+	+	+	+	1,800 fold
	Third	14	−	+	+	+	+	+	+	+	+	7,200 fold
	Fourth	14	−	−	+	+	+	+	+	+	+	3,600 fold

− Signifies no visible growth
+ Signifies visible growth

TABLE 4

CROSS-RESISTANCE BETWEEN ISONIAZID- AND VERAZIDE-RESISTANT MUTANTS OF H37Rv

Medium: Serum-synthetic, 14 days' incubation at 37°C.

Mutant	Tuberculostatic End Point with	
	Isoniazid	Verazide
	IM	*IM*
Isoniazid-resistant	2,000 (68 γ per ml.)	2,000 (142 γ per ml.)
Verazide-resistant	1,000 (137 γ per ml.)	1,000 (284 γ per ml.)
Wild-type H37Rv	2,048,000 (0.07 γ per ml.)	4,096,000 (0.07 γ per ml.)

In vivo antituberculous activity: As already indicated, the technique of *in vivo* screening used was previously standardized healing ulcer method (8), which facilitates the evaluation of activity rapidly and objectively. Complete healing of tuberculous ulceration within twenty-eight days, which is the aim of the treatment, is recorded as H.R. 100, but it is not implied that this value means complete elimination of the bacillary population in the treated animal. The correlation between the ulcer response and that of other tuberculous tissues is generally very reliable, as indicated by some typical results listed in table 5. It is evident that the H. R. response is a useful means of evaluating and expressing the relative activity of isoniazid, Verazide, and other hydrazones.

In figures 2 and 3, a typical *in vivo* experiment is recorded in which a comparison between the activity of isoniazid and Verazide is given for various dosage regimens. It will be immediately obvious in these graphs (and from table 6) that no significant difference in activity is detectable when the two drugs are given in low daily dosage. However, sharp differences emerged when the dosage was increased but given at widely spaced time intervals. For example, Verazide was markedly more active than isoniazid as the intermittency

TABLE 5

COMPARISON OF SEVERITY OF THE TUBERCULOUS PROCESS WHEN ASSESSED BY MEASUREMENT OF ULCERS AND GRADING OF INTERNAL ORGANS

Animal Number	Drug	Average Diameter of 4 Ulcers	Grading of Internal Organs*				Correlation
			Spleen	Liver	Lung	Combined	
		mm.					
1	No treatment (controls)	7	3	2	3	8	Good
2		13	3	3	3	9	Good
3		13 (10)	4	3	3	10 (9)	Good
4		11	3	1	2	6	Fair
5		9	4	3	3	10	Good
6		9	3	3	3	9	Good
7	Verazide 30 mg./kg. weekly	1	2	1	2	5	Fair
8		0	0	0	1	1	Good
9		0 (<1)	0	0	1	1 (2)	Good
10		0	0	0	1	1	Good
11	Isoniazid 30 mg./kg. weekly	1	1	0	0	1	Good
12		0	1	0	0	1	Good
13		0 (<1)	0	0	1	1 (1)	Good
14		0	0	0	0	0	Good
15	Verazide 60 mg./kg. fortnightly	0	1	0	0	1	Good
16		3	1	2	2	5	Good
17		1 (3)	0	0	0	0 (2)	Good
18		9	0	1	2	3	Poor
19	Isoniazid 5 mg./kg. daily	0	2	0	0	2	Good
20		0	1	0	1	2	Good
21		0 (<1)	0	0	0	0 (1)	Good
22		0	0	0	0	0	Good

* The numbers indicate the degree of tuberculous involvement as one-plus, two-plus, et cetera.

of dosage was increased to fortnightly or monthly treatment, the latter representing only a single dose given during the experimental observation period.

In table 6 the H.R. values at twenty-one and twenty-eight days of treatment are recorded (some of which have been calculated from figures 2 and 3). They confirm the conclusion that Verazide exerts a more prolonged action than isoniazid (*cf. Experiments 7* and *17*, *10*, and *19*) and require little elaboration here. It may be remarked, however, that Verazide, while active when given orally, is less effective by this route than by injection (*Experiments 5* and *12*). This is probably due to malabsorption from the intestine which, in turn, may be determined by its low solubility. The findings suggest that the minimal effective dose in the treatment of guinea pig tuberculosis is 20 mg. per kg. of body weight per week by the intramuscular route (*Experiment 5*) or the same dose twice weekly by mouth (*Experiment 12*).

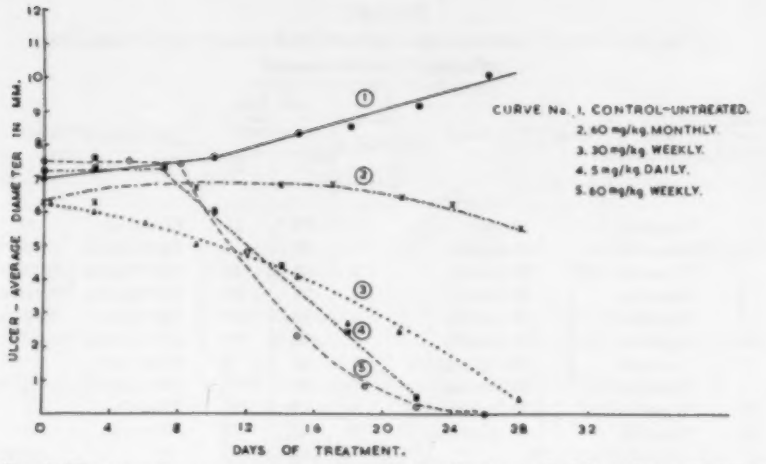
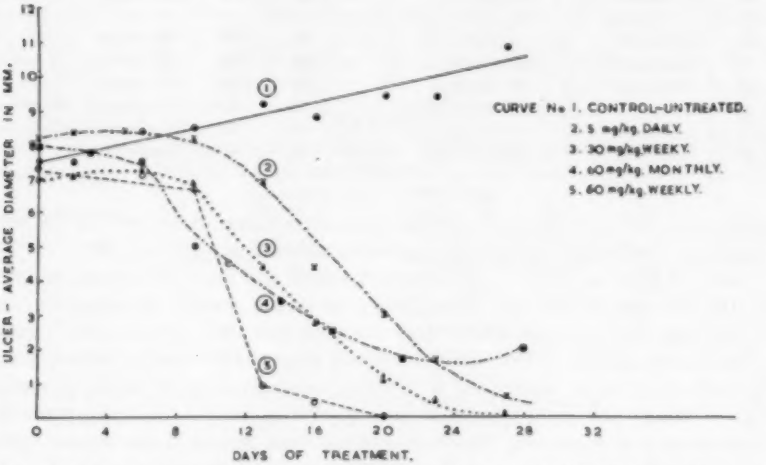

ULCER - AVERAGE DIAMETER IN MM.
DAYS OF TREATMENT.
CURVE No. 1. CONTROL-UNTREATED.
2. 60 mg/kg. MONTHLY.
3. 30 mg/kg. WEEKLY.
4. 5 mg/kg. DAILY.
5. 60 mg/kg. WEEKLY.

FIG. 2. The rate of response of tuberculous ulcers when treated with various regimens of isoniazid by intramuscular injection.


ULCER - AVERAGE DIAMETER IN MM.
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2. 5 mg/kg. DAILY.
3. 30 mg/kg. WEEKLY.
4. 60 mg/kg. MONTHLY.
5. 60 mg/kg. WEEKLY.

FIG. 3. The rate of response of tuberculous ulcers when treated with various regimens of Verazide by intramuscular injection.

Comparison of Verazide and related hydrazones: A comparison may now be made between Verazide and some closely related hydrazones. In table 7 are recorded the results in which it may be seen that, of the six hydrazones examined, Verazide and its 3:4-dihydroxy analogue (protozide) were the most active. The 2-hydroxybenzaldehyde (Salizid) and vanizide (Phtivazid) were measurably less active than Verazide. Nitrozide, the 4-nitrobenzaldehyde

TABLE 6

IN VIVO ACTIVITY OF VERAZIDE AND ISONIAZID IN TUBERCULOUS GUINEA PIGS
(Healing Ulcer Technique)

Experiment Number	Drug	Route	Dosage	Number of Animals	H.R. Value at — Days of Treatment		Comment on Treatment
					21	28	
			mg. per kg.				
1	Verazide	Intramuscular	5 daily	4	73	94	Effective
2	Verazide		5 weekly	4	12	15	Ineffective
3	Verazide		10 weekly	4	37	58	Moderately effective
4	Verazide		15 weekly	4	39	61	Moderately effective
5	Verazide		20 weekly	4	86	100	Effective
6	Verazide		30 weekly	4	85	98	Effective
7	Verazide		30 fortnightly	8	53	73	Effective
8	Verazide		45 fortnightly	4	78	92	Effective
9	Verazide		60 fortnightly	8	32	87	Effective
10	Verazide		60 monthly	8	63	77	Effective
11	Verazide	Oral	15 weekly	4	39	51	Moderately effective
12	Verazide		20 twice weekly	4	86	100	Effective
13	Verazide		30 weekly	4	64	78	Effective
14	Isoniazid	Intramuscular	5 daily	4	89	100	Effective
15	Isoniazid		20 weekly	4	68	100	Effective
16	Isoniazid		30 weekly		72	95	Effective
17	Isoniazid		30 fortnightly	8	33	60	Moderately effective
18	Isoniazid		60 fortnightly	8	30	59	Moderately effective
19	Isoniazid		60 monthly	8	32	41	Ineffective

hydrazone, was quite inactive even at doses three times those used for the other hydrazones, although it could not be distinguished from them *in vitro*.

Sah and Peoples (3) have also screened Salizid and vanizide in mice at a dose of 0.05 per cent in the diet. They found, as in the present investigation, that Salizid was slightly more active than vanizide, but they did not test Verazide or protozide, which, in this series, were the most active chemotherapeutically. In view of the close agreement with drugs common to both series, it appears that Verazide and protozide must be regarded as two of the most promising hydrazones yet examined. Vanizide was the least active of the active hydrazones tested.

Verazide-companion drug regimens: In view of the interest in Verazide as the isoniazid hydrazone of choice in this particular study, the response of guinea pigs to combined therapy was measured. In these experiments Verazide was paired with isoniazid, streptomycin, and *p*-aminosalicylic acid (PAS). In addition, the influence of pyridoxine on chemotherapeutic activity was studied. The results are listed in table 8.

The results can be summarized (table 8) by stating that no enhancement or interference of Verazide action was detectable in these short-term animal experiments with subeffective doses of streptomycin or PAS or with pyridoxine in

TABLE 7

COMPARISON OF IN VIVO ACTIVITIES OF VARIOUS HYDRAZONES OF ISONIAZID IN TUBERCULOUS GUINEA PIGS
(Healing Ulcer Technique)

Drug	Formula (*see* figure 1)	Route	Dosage	Number of Animals	H.R. Value at — Days of Treatment		Comment on Treatment
					21	28	
			mg. per kg.				
Verazide	I	Intramuscular	10 weekly	4	37	58	Moderately effective
			20 weekly	4	86	100	Effective
Vanizide (Phtivazid)	II		10 weekly	6	0	0	Ineffective
			20 weekly	6	34	57	Moderately effective
Orthovanizide	III		10 weekly	6	16	27	Ineffective
			20 weekly	6	36	49	Ineffective
Salizid	IV		10 weekly	6	8	7	Ineffective
			20 weekly	6	43	62	Moderately effective
Protozide	V		10 weekly	6	19	32	Ineffective
			20 weekly	6	45	83	Effective
Nitrozide	VIII		60 weekly	4	8	12	Ineffective

TABLE 8

IN VIVO ACTIVITY OF VERAZIDE IN COMBINATION WITH OTHER ANTITUBERCULOUS AGENTS AND PYRIDOXINE
(Healing Ulcer Technique)

Experiment Number	Drug Combination	Dosage and Route	Number of Animals	H.R. Value at — Days of Treatment	
				21	28
		mg. per kg.			
1	Verazide only	20 weekly, intramuscular	4	86	100
2	Isoniazid only	20 weekly, intramuscular	4	68	100
3	Streptomycin only	10 twice weekly, intramuscular	4	15	36
4	*p*-Aminosalicylic acid only	200 daily, oral	4	13	10
5	Verazide and isoniazid	as above	4	95	100
6	Verazide and streptomycin	as above	4	88	100
7	Verazide and *p*-aminosalicylic acid	as above	4	85	100
8	Verazide and pyridoxine HCl (10 mg. daily intramuscularly)	as above	4	56	98

high daily dosage. The isoniazid-Verazide combination was particularly active but, as each drug was given in therapeutically effective doses, no conclusion can be drawn from *Experiment 5* except that the two drugs do not act as antagonists to each other. In passing, it might be noted that PAS was particularly

TABLE 9

IN VIVO ACTIVITY OF SOME METAL COMPLEXES OF ISONIAZID AND VERAZIDE
(Healing Ulcer Technique)

Experiment Number	Complex	Dosage and Route	Number of Animals	H.R. Value at — Days of Treatment	
				21	28
		mg. per kg.			
1	Isoniazid-$FeSo_4 \cdot 2H_2O$	20 weekly, intramuscular	4	42	80
2	Isoniazid-$FeSO_4 \cdot 2H_2O$	20 twice weekly, oral	4	84	99
3	Isoniazid-$CoSo_4 \cdot 2H_2O$	20 weekly, intramuscular	4	46	62
4	Isoniazid-$ZnSo_4 \cdot 2H_2O$	20 weekly, intramuscular	4	70	99
5	Isoniazid-$CuSo_4 \cdot 2H_2O$	20 weekly, intramuscular	4	85	99
6	Verazide-$CuCl_2 \cdot 2H_2O$	20 weekly, intramuscular	4	86	93
7	Isoniazid (for comparison)	20 weekly, intramuscular	4	68	100
8	Verazide (for comparison)	20 weekly, intramuscular	4	86	100

TABLE 10

ACUTE TOXICITY (LD_{50}) OF ISONIAZID, VERAZIDE, AND OTHER HYDRAZONES
(Mice Injected by Intraperitoneal Route)

Complex (*see* figure 1)	Drug	LD_{50} Mg. per Kg. Body Weight	Toxicity When Compared with Isoniazid
I	Verazide	700	3.5 fold reduction
III	Orthovanizide	1,000	5 fold reduction
VI	Piperazide	480	4.5 fold reduction
VII	Benzalide	800	4 fold reduction
II	Vanizide (Phtivazid)	>20,000 (impossible to inject higher dose)	>100 fold reduction
IV	Salizid	5,500	28 fold reduction
V	Protozide	6,500	33 fold reduction
	Isonizid	200	standard
	Isoniazid-$CuSO_4 \cdot 2H_2O$	40	5 fold increase

ineffective even at a dosage of 200 mg. per kg. daily, that is, approximately sixty times greater than the minimal effective dose of Verazide.

Finally, the metal complexes were examined for *in vivo* activity by giving suspensions of the various complexes at weekly intervals intramuscularly. The results are shown in table 9.

In general, all of the complexes, with the exception of the cobalt derivative, were as active as the parent drug. However, the local toxicity of some of the derivatives was so severe that treatment had to be discontinued (figure 4).

Acute toxicity of various hydrazones—LD_{50} in mice: The acute toxicity of the various derivatives described above was determined by intraperitoneal injection into mice of the average weight of 20 gm. Groups of 12 or more weighed mice received various doses (at least five) of the test substance, and were ob-

FIG. 4. Necrotic ulceration induced by three intramuscular injections of the copper complexes of Verazide (left) and isoniazid (right) containing approximately 8 mg. of complex suspended in 0.4 ml. of saline. Note the healed tuberculous ulcer on the animal on the left, indicating antituberculous activity.

served over a period of seven days. The LD_{50} was calculated from a graph in which the log of the dose was plotted against percentage mortality.

The results are recorded in table 10. It will be noted that the variation in acute toxicity extends over an extraordinarily wide range from the copper complex of isoniazid, which is particularly toxic (LD_{50} = 40 mg. per kg.), to the 3-methoxy-4-hydroxybenzal hydrazone, vanizide, which is virtually nontoxic (LD_{50} > 20,000 mg. per kg.). The hydrazones fall into two broad groups, the nontoxic group having a thirty fold or greater reduction in toxicity when compared with isoniazid (vanizide, Salizid, and protozide), and the slightly toxic group showing a fourfold reduction in toxicity (Verazide, orthovanizide, piperazide, and benzalide).

Discussion

The main purpose of this paper has been to describe in detail the antituberculous activity of Verazide, together with some closely related hydrazones of isoniazid. The present writers had previously indicated that Verazide was a drug of potential value in the treatment of human tuberculosis, and this study and the accompanying paper (10) confirm this view.

The results can be summarized briefly in the following way. The *in vitro* activity of the hydrazones of isoniazid is usually equal to that of isoniazid itself (table 1), and this activity is unaffected by serum proteins. Of the hydrazones examined, Verazide appeared to be slightly more active *in vitro* than the others, but the difference was probably not significant. The *in vivo* studies, however, revealed differences not detectable *in vitro* especially when careful attention to dosage regimens was exercised. For example, it was possible to demonstrate significant differences in chemotherapeutic activity when doses of 10 and 20 mg. per kg. of body weight were given intramuscularly at weekly intervals. It may be seen (table 7) that this two-dose test effectively distinguished *in vivo* activity of the different hydrazones which would have otherwise been masked by giving a higher total dosage. Verazide was obviously the most active member of the series studied, which included two hydrazones of some clinical interest, vanizide (Phtivazid in USSR), and Salizid. These two hydrazones were only moderately effective at a dosage of 20 mg. per kg., the H.R. values being about 60, and quite ineffective at the 10 mg. per kg. dose. By comparison, Verazide had approximately twice the *in vivo* activity of either derivative.

When the *in vivo* activity of Verazide is compared with that of isoniazid (table 6 and figures 2 and 3), there seems little to distinguish the two compounds. At a low daily dosage (5 mg. per kg.), both are equally effective; at weekly dosage, both are similarly active; at fortnightly and monthly dosage, however, Verazide is slightly more effective. This is brought out more clearly in figures 2 and 3, in which it is apparent that the rate of reversal of the tuberculous process is more rapid with Verazide than with isoniazid when given intermittently. These observations are more impressive when it is remembered that Verazide has approximately twice the molecular weight of isoniazid, so that, if the comparison is made on a molar basis, Verazide has twice the activity of isoniazid. Several reasons can be suggested for this, e.g., higher tissue concentration, delayed excretion, resistance to chemical acetylation, and similar phenomena, but these cannot be discussed profitably in the absence of experimental evidence.

An attempt was made to measure the influence of subeffective doses of streptomycin and PAS on Verazide therapy. No beneficial change could be detected (table 8), although the short-term nature of the experiment precluded observation of anything but a dramatic stimulation of Verazide activity. On the other hand, there was no evidence of any deleterious action by either of these adjuvants or by high daily doses of pyridoxine (table 8).

The low acute toxicity of these hydrazones of isoniazid (table 10) once more emphasizes the importance of these derivatives as potentially interesting chemo-

therapeutic agents. This topic will be expanded in a paper immediately following (10). Briefly, they can be grouped into two classes: those exhibiting an extremely low toxicity and those showing a moderately low toxicity. Verazide falls in the second group, whereas Salizid and vanizide belong to the former. However, the markedly higher *in vivo* activity of Verazide appeared to be a more important attribute for clinical recommendation than an extraordinarily low toxicity. In fact, protozide, the 3:4-dihydroxybenzal hydrazone, is one of the most chemotherapeutically interesting derivatives of the very low toxicity group. Further work on this derivative is contemplated.

The emergence of Verazide-resistant catalase-negative mutants *in vitro* is easily accomplished (table 3), as is the case with isoniazid. Complete cross-resistance can also be readily demonstrated between Verazide- and isoniazid-resistant strains (table 4), indicating that the mode of action of the two drugs is similar. Whether resistant forms will emerge more rapidly with Verazide therapy than with isoniazid cannot be predicted from the evidence available.

The high *in vitro* and *in vivo* activity of the metal complexes of isoniazid and the copper complex of Verazide has been demonstrated. However, none of these derivatives could be considered seriously in the chemotherapy of tuberculosis because of their high local and acute toxicity (figure 4, table 10).

In conclusion, it has been shown that the therapeutic index of isoniazid can be increased by hydrazone formation, this effect being due to a lowering of acute toxicity without a corresponding reduction of antituberculous activity. Accordingly, these derivatives must be seriously considered in the progress of chemotherapy in tuberculosis. Of the derivatives which the writers have had the opportunity of examining, Verazide (3:4-dimethoxybenzal hydrazone of isoniazid) is the drug of choice.

Summary

In vitro and *in vivo* studies of isoniazid, Verazide, and related hydrazones have been described. It has been shown that the *in vitro* activity of hydrazone derivatives of isoniazid is equal to that of the parent substance, isoniazid. By using a low critical dosage, 10 mg. per kg. of body weight weekly by intramuscular injection, it was possible to demonstrate differences in chemotherapeutic activity of the various hydrazones. The results of these and other tests showed that Verazide exerted a more marked and more prolonged antituberculous activity than did isoniazid or the other hydrazones. In view of this and of the fact that the hydrazones generally exhibit a significantly lower toxicity than isoniazid, it is considered that some of these derivatives would be more suitable chemotherapeutic agents for the treatment of human tuberculosis than isoniazid itself. Of the hydrazones examined here and by others (2–6), it appears that Verazide is the drug of choice for clinical application.

The mode of action against the tubercle bacillus exhibited by Verazide and isoniazid is of the same type. Resistant mutants are readily isolated which show complete cross-resistance.

Finally, the metal complexes of isoniazid and the copper complex of Verazide

have been studied *in vitro* and *in vivo*. All showed marked antituberculous activity and a high local and acute toxicity; hence they cannot be considered as potential chemotherapeutic agents.

Sumario

Quimioterapia de la Tuberculosis. I. Actividad Antituberculosa de la Veracida y las Hidrazonas Afines

Preséntanse estudios *in vitro* e *in vivo* de la isoniacida, la veracida y las hidrazonas afines. Queda demostrado que la actividad *in vitro* de los derivados hidrazónicos de la isoniacida es igual a la de la substancia matriz, la isoniacida. Usando una dosis crítica baja, de 10 mg. por kg. de peso semanalmente en inyección intramuscular, fué posible demostrar las diferencias que había en la actividad terapéutica de las varias hidrazonas. Los resultados de estos y de otros ensayos revelaron que la veracida ejercía una acción antituberculosa más intensa y prolongada que la de la isoniacida o de las demás hidrazonas. En vista de esto y del hecho de que las hidrazonas manifiestan generalmente una toxicidad decididamente menor que la de la isoniacida, se cree que algunos de estos derivados serían agentes terapéuticos más adecuados para la tuberculosis humana que la isoniacida misma. De las hidrazonas estudiadas aquí y por otros (2-6), parece que la veracida es la droga de elección para aplicación clínica.

El modo de acción contra el bacilo tuberculoso mostrado por la veracida y la isoniacida es del mismo género, siendo fácil aislar mutantes resistentes que despliegan absoluta resistencia cruzada.

Por fin, se han estudiado *in vitro* e *in vivo* los complejos metálicos de la isoniacida y el complejo cúprico de la veracida. Todos revelaron notable actividad antituberculosa y elevada toxicidad local y aguda; de ahí que no puedan considerarse como potenciales agentes quimioterapéuticos.

Resume

Chimiothérapie de la tuberculose: I. Activité antituberculeuse de la Vérazide et des dérivés hydrazones

Les études *in vitro* et *in vivo* de l'isoniazide, de la vérazide et des hydrazones apparentés sont décrites. Il a été démontré que l'activité *in vitro* des dérivés hydrazones de l'isonazide est égale à celle de la substance mère, l'isoniazide. En utilisant la dose active minima de 10 mg par kg de poids corporel en injection intramusculaire hebdomadaire, il a été possible de démontrer des différences dans l'activité chimiothérapeutique des diverses hydrazones. Les résultats de ces tests et d'autres épreuves ont montré que la Vérazide exerçait un effet antituberculeux plus prolongé que celui produit par l'izoniazide ou les autres hydrazones. Compte tenu de ces résultats et du fait que la toxicité des hydrazones se montre généralement nettement plus basse que celle de l'isoniazide, les auteurs estiment que certains de ces dérivés constitueraient des agents chimiothérapeutiques plus favorables dans le traitement de la tuberculose humaine que l'isoniazide elle-même. Parmi les hydrazones examinées dans cette étude et par d'autres auteurs (2-6), il apparaît que la vérazide est le médicament de choix en clinique.

Le mode d'action contre le bacille tuberculeux exercé par la vérazide et l'isoniazide sont du même type. Il est facile d'isoler des mutants résistants doués d'une résistance croisée complète.

Enfin, les composés métalliques de l'isoniazide et la combinaison de la vérazide avec le cuivre ont été étudiés *in vitro* et *in vivo*. Ils ont tous témoigné d'une activité antituberculeuse marquée et d'une toxicité aiguë et locale élevée; en conséquence, ils ne peuvent être envisagés comme des agents chimiothérapeutiques éventuels.

Acknowledgments

The writers wish to thank the National Health & Medical Research Council, the contributors to the Argus Research Fund, and Dr. A. E. Rowden White, C.M.G., for generous financial assistance. Also their thanks are due to their colleague, Dr. J. Cymerman-Craig, for helpful assistance in the chemical section of this paper and for preparing and supplying nitrozide.

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CHEMOTHERAPY OF TUBERCULOSIS[1]

II. Some Observations on the Pharmacology of Verazide

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INTRODUCTION

In the preceding paper (1) the antituberculous activity of Verazide, 3:4-dimethoxybenzal isonicotinyl hydrazone was described. Among other things, it was shown that: Verazide is as active as isoniazid *in vitro*; Verazide is more active than isoniazid *in vivo* when given in intermittent dosage; and Verazide is three to four times less toxic than isoniazid. These findings, and the fact that Verazide is not inactivated *in vivo* by acetylation and cannot chemically combine with pyridoxal to induce a peripheral neuritis, led to the recommendation that it should be eminently suitable for the treatment of human tuberculosis.

The present study deals with those aspects of the pharmacology of Verazide which would provide information essential for a clinical trial of the drug. The results to be described indicate that Verazide can be used safely in man in doses approximately four times those recommended for isoniazid. At such a dose, 20 mg. per kg. of body weight daily, the Verazide concentrations in the blood fluctuate between ten and forty times the concentration required to inhibit the growth of tubercle bacilli. It would appear, therefore, that Verazide should be an effective and safe chemotherapeutic agent. These predictions are now being tested in clinical trials which have commenced in Australia and South Korea. The results will be published at a later date.

MATERIALS AND METHODS

Chemical estimation: The estimation of isoniazid and Verazide in biologic fluids and tissues was carried out, using extraction and spectrophotometric measurement. Isoniazid was estimated by the method of Kelly and Poet (2) while Verazide was measured by the method of Rubin, Drekter, Scheiner, and De Ritter (3).

Another procedure for the estimation of Verazide, which correlated well with the above, was to extract 3 ml. of the biologic fluid (or tissue homogenate) three times with 2 ml. volumes of ethyl acetate. By measuring the absorption at a wavelength of $\lambda = 330$ mμ of the combined ethyl acetate extracts, it was possible to calculate the concentration of Verazide ($E_1^1 = 780$ at $\lambda = 330$ mμ) using as a control ethyl acetate extracts of normal fluids and tissues. For purposes of standardization, known amounts of Verazide were added to normal tissues and extracted as above. The amounts recovered were then used as standard recovery figures. The method was found to be accurate (90 per cent recovery) and convenient, since the ultraviolet absorption of biologic fluids is very small in the region of maximal absorption of Verazide. The lowest concentration of measurable Verazide by this procedure was found to be 2γ per ml. of serum or per gm. of tissue. For the estimation of Verazide in cerebrospinal fluid, a direct spectrophotometric measurement could be used.

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Animal toxicity: The acute systemic toxicity (LD_{50}) of Verazide in mice has been determined previously (4). The systemic toxicity reported here is restricted to chronic toxicity tests in guinea pigs and acute toxicity in dogs and rabbits, with the view of getting an indication of the order of tolerable dosage regimens in humans.

Local tissue toxicity was assessed histologically by examination of muscle tissues of guinea pigs into which Verazide had previously been injected.

Human toxicity: Five seriously ill volunteer patients in a hospital (the Austin Hospital) were given Verazide by mouth in increasing dosage over a period of six months, and their response to the drug was assessed by clinical, biochemical, and hematologic techniques.

Results

Stability of Verazide

Verazide is sparingly soluble in water (1 in 3,000 at 20°C.), but readily soluble in dilute acid solution, in which it is hydrolyzed to isoniazid and veratric aldehyde (3:4-dimethoxy-benzaldehyde).

The rate and degree of hydrolysis at different pH levels were determined in a solution containing 12 γ per ml. in 50 per cent alcohol, and adjusted with dilute hydrochloric acid to pH levels ranging from 1.1 to 6.0. These solutions were incubated at 37°C. and examined spectrographically, together with 50 per cent alcohol containing hydrochloric acid as a control, at various time intervals. From the absorption spectra the rate of hydrolysis of Verazide was calculated. The results have been recorded in figure 1.

It may be seen that below pH 2.4 Verazide is 50 per cent hydrolyzed in thirty minutes, but above pH 3.5 the drug is stable for at least six hours.

The stability in gastric juice was also studied, using material from human, monkey, and guinea pig sources. In general the results, shown in table 1, were the same as those obtained in 50 per cent acidified alcohol at the same pH.

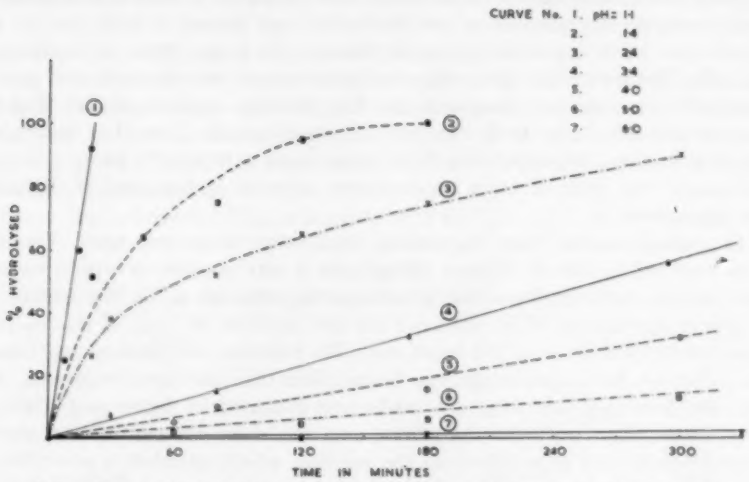

Fig. 1. Rate of hydrolysis of Verazide in 50 per cent aqueous alcohol at 37°C.

TABLE 1

STABILITY OF VERAZIDE IN GASTRIC JUICE AT 37°C.

Source of Gastric Juice	pH	Per Cent Veraside Hydrolyzed at — Minutes					
		0	20	80	120	360	∞
Human	1.9	0	41	81	90	100	100
	2.3	0	28	60	72	94	100
Guinea pig	3.5	0	—	19	27	—	87
Monkey	4.5	0	0	—	11	—	42

Thus, it would appear that Verazide is likely to be hydrolyzed to isoniazid when given by mouth, particularly if the emptying time of the stomach is longer than three hours. This, however, is not necessarily the case, since most of the hydrazones, when given as a compressed tablet, are not exposed to acid hydrolysis and the emptying time of the stomach is usually a half hour to one hour. That the degree of hydrolysis does not seriously interfere with the assimilation of Verazide as such will be demonstrated later (figure 3A).

Tolerance of Verazide on Intramuscular Injection

In the beginning it was hoped that Verazide might be used by depot-dosing injection as in the guinea pig experiments (1). In these, Verazide was given by intramuscular injection, usually in doses of 20 mg. per kg. of body weight at weekly intervals in a dilute suspension containing 20 mg. per ml. of saline. Such a suspension is too dilute for human use (approximately 70 ml. would be required for a 70 kg. person) and obviously had to be at least twenty to thirty times more concentrated.

When these concentrated suspensions were prepared, a curious phenomenon was observed—the suspension lost its fluidity and formed a thick viscous gel which was quite impossible to inject through the larger types of needle e.g., No. 16*g*. The reason for this unexpected phenomenon was demonstrated microscopically. In aqueous suspension the flat platelike crystals of the drug reformed into fine long needle-like crystals which quickly formed a continuous sheet of solid in the suspending fluid. After many attempts to prevent recrystallization, the idea of using concentrated aqueous suspensions of Verazide was abandoned.

In seeking another inert suspending fluid, ethyl oleate was tried. Verazide dispersed well in this oil without change, and it was possible to expel a suspension of 1 gm. in 3 ml. (sterilized by autoclaving) through a No. 20*g* needle.

One of the writers (SDR) received the first injection of 1 ml. of this suspension intramuscularly as a trial depot dose. The injection was painless and caused no discomfort for about twenty-four hours. After this time deep tenderness over the site developed, and later the tenderness increased in degree and distribution. By seventy-two hours the gluteal area was distinctly painful and the recipient was doubly miserable from the reaction, which subsided in seven to ten

days. This unpleasant experience made it quite obvious that Verazide could not be given to humans by injection. Subsequent experiments in guinea pigs provided the reason for this result.

Systemic Animal Toxicity

Mice: The acute toxicity (LD_{50}) of Verazide for mice has been previously reported (1) as 700 mg. per kg. of body weight in contrast to an LD_{50} of isoniazid of 200 mg. per kg. Chronic toxicity in mice was studied by intraperitoneal injection of 60 mg. per kg. of body weight daily, that is, three times the anticipated daily dose in humans. The treatment extended over a period of four weeks, after which all of the mice were sacrificed and the tissues were examined macroscopically and microscopically. No evidence of toxicity could be detected.

One aspect of toxicity which might be considered relevant to the assessment of Verazide and related hydrazones is the toxicity of the aldehydes from which they are derived and which may be liberated by hydrolysis in the stomach. The LD_{50} of aldehydes from four potentially interesting hydrazones was determined by intraperitoneal injection into mice. The results are shown in table 2 below.

It will be noted that veratric aldehyde had much the same LD_{50} as its hydrazone Verazide, 750 and 700 mg. per kg. of body weight, respectively, whereas the other aldehydes were considerably more toxic than their corresponding isonicotinyl hydrazones.

Rabbits: Intravenous injection of 2 to 3 ml. of an aqueous suspension of Verazide (20 mg. per ml.) in rabbits caused immediate death, but when the same dose was given in a more dilute suspension (10 mg. per ml.) no ill effect was observed. The sudden convulsive death with concentrated suspensions at very low dosage (20 mg. per kg.) and the innocuousness of dilute suspensions clearly indicated that this toxic reaction was embolic and not chemical in nature. Much larger doses could be given intravenously in ethyl oleate without producing embolic or toxic symptoms. However, these findings indicated that intravenous injection of concentrated suspensions of Verazide carried the danger of embolic complications.

Dogs: Dogs tolerated Verazide in doses of 20 mg. per kg. when given intrave-

TABLE 2

ACUTE TOXICITY (LD_{50}) OF AROMATIC ALDEHYDES OF SOME ISONICOTINYL HYDRAZONES

Aldehyde	LD_{50}	LD_{50} of Corresponding Isonicotinyl Hydrazone (1)
	mg. per kg. body weight	
Salicylaldehyde	200	5,500
Protocatechuic aldehyde	500	6,500
Vanillin	600	>20,000
Veratric aldehyde	750	700

nously and 100 mg. per kg. intramuscularly. One animal was given 600 mg. per kg. intramuscularly. Severe swelling of the leg occurred and seven days later the dog died. The cause of death was not obvious.

Local Tissue Toxicity

As indicated earlier, the local tissue toxicity was determined by examining sections of injected muscle tissue stained by hematoxylin-eosin. Suspensions of different strengths in saline and ethyl oleate were used and the results, which were uniformly consistent, can be summarized briefly as follows.

Three to four days after the injection of 0.2 ml. of the ethyl oleate suspension intended for human use (originally 330 mg. per ml.), an acute inflammatory response developed around the deposited drug. Polymorphonuclear cells predominated, blood vessels were distended, and the muscle tissue showed evidence of necrosis. At seven days there was typical abscess formation at the site and evidence of fibrosis. By ten days fibrous scar tissue had formed in the muscle bundle, and healing was complete by about the fourteenth day.

With weaker suspensions the inflammation and necrosis were less marked, and with the dilute suspensions used for guinea pig experiments (20 mg. per ml.) the degree of damage was negligible.

It might be added that other hydrazones of isoniazid (protozide and vanizide) were similarly tested and found to excite the same type of response. It appears that these sparingly soluble hydrazones, which might be considered useful for depot dosing on theoretical grounds, are unsuitable for this purpose.

Chronic Toxicity in Humans

Five volunteers, 3 suffering from chronic bone tuberculosis and 2 from advanced pulmonary tuberculosis, were given Verazide orally in enteric-coated tablets over a period of six months. During the first two months the dose was 10 mg. per kg. of body weight, in the second two months this was increased to 15 mg. per kg., and for the last two months 20 mg. per kg. were given. The following summaries have been prepared by Dr. Peter Bull (clinician) and Dr. Noel Orchard (pathologist), under whose supervision this chronic toxicity trial was conducted.

Clinical Summary (Dr. Peter Bull)

Patient P.C., aged 46, was suffering from gross ankylosing spondylitis, anal fistula, and sinus from the right hip, all of tuberculous origin. A chest roentgenogram showed quiescent pulmonary tuberculosis. During the trial the sinus closed and remained closed for the longest period since 1953. There was no evidence of Verazide toxicity, although the patient complained from time to time of a burning feeling in the mouth after taking the tablets and also after taking a placebo.

Patient A. E., aged 64, had tuberculosis of the spine and paraplegia, but without deformity or sinuses. She showed no change during the trial and no evidence of toxicity.

Patient J.H., aged 66, was suffering from gross bilateral pulmonary tuberculosis with cavitation and sputum containing isoniazid-resistant tubercle bacilli. His mental state was not quite "of this world." During and at the end of the trial his clinical condition was

unchanged, although he stated that he felt much better. There was no evidence of drug toxicity.

Patient W.B., aged 48, had gross bilateral pulmonary tuberculosis with sputum containing tubercle bacilli resistant to all chemotherapeutic agents. He had extreme dyspnea, and 250 to 350 ml. of purulent sputum were expectorated daily. During the trial of the drug there was no change in the patient's condition until the fifth month when he developed edema. A few days after the end of the trial the patient died. Autopsy showed cor pulmonale, gross congestive cardiac failure, and bilateral cavitation in the lungs. This patient was very sick when he entered the trial, and death had been anticipated earlier. There was no evidence of drug toxicity.

Patient N.A., aged thirty, at the commencement of the trial of the drug had bilateral tuberculosis of the hips with intermittently discharging sinuses, tuberculosis of the spine, paraplegia, and gross deformity. During the drug trial the sinuses ceased to discharge; otherwise there was no evidence of change. The temperature chart was unchanged; there was no evidence of neuritis or of any other toxic manifestation. Since Verazide has been discontinued, the sinuses have started to discharge again but the general condition is unchanged.

Summarising, none of the patients showed any clinical evidence of toxicity, in two cases the bone sinuses closed and one patient died soon after completion of the trial. His death could not be attributed to the use of Verazide.

Clinicopathologic summary (Dr. Noel Orchard)

Before the trial the following investigations were carried out on the 5 patients: serum proteins (total and differential), cephalin flocculation, serum bilirubin, serum alkaline phosphatase (reported as King-Armstrong units), blood sugar, blood urea, examination of the urine for albumin, casts and cells, erythrocyte sedimentation rate, hemoglobin, leukocyte and differential blood counts, and an examination of the blood smear. During the first three months of the trial a hematologic investigation was made weekly and a full investigation, once a fortnight. Following this, a complete investigation was carried out once a month during the trial and for one month thereafter.

The results, summarized in table 3, showed no marked changes in any of the biochemical tests, although all of the patients developed a transient absolute eosinophilia at some stage during the trial. In short, the biochemical and hematologic tests failed to show any evidence of toxicity.

Concentration of Verazide in Serum

The concentration of Verazide in serum was determined in healthy subjects, mainly members of the staff and students, who were given the drug by mouth either as enteric-coated or uncoated tablets. The results are shown in figures 2 and 3.

It may be seen in figure 2 that the serum concentrations following single doses of enteric-coated tablets at different dosages followed the same pattern. Four hours after administration the Verazide concentration reached its peak and remained at this level for about two hours, after which it slowly fell over a period of six to twelve hours, depending on the dose given, to a chemically nondetectable concentration.

It is interesting to note in figure 2 that at a dose of 5 mg. per kg. the highest concentration of Verazide in serum was 2 γ per ml. after four hours and that the peak concentrations with 10 mg. per kg. and 20 mg. per kg. doses were 3.2

TABLE 3

SUMMARY OF CLINICOPATHOLOGIC INVESTIGATIONS FROM CHRONIC TOXICITY TRIAL OF VERAZIDE

Patient Investigation	P.C. (Age 46)				A.E. (Age 64)				J.H. (Age 66)				W.B. (Age 48)				N.A. (Age 30)			
	Before	Verazide Dose			Before	Verazide Dose			Before	Verazide Dose			Before	Verazide Dose			Before	Verazide Dose		
		10 mg./kg.	*15 mg./kg.*	*20 mg./kg.*		*10 mg./kg.*	*15 mg./kg.*	*20 mg./kg.*		*10 mg./kg.*	*15 mg./kg.*	*20 mg./kg.*		*10 mg./kg.*	*15 mg./kg.*	*20 mg./kg.*		*10 mg./kg.*	*15 mg./kg.*	*20 mg./kg.*
Serum proteins, %	7.4	6.5	6.8	6.5	6.8	5.6	5.3	5.6	6.8	5.2	6.1	6.6	6.6	6.8	6.8	6.7	6.0	6.5	6.0	6.3
Serum albumin, %	3.8	3.4	4.0	3.6	4.7	4.4	4.0	4.1	3.3	3.0	3.1	3.3	4.2	4.0	4.5	4.2	4.7	4.3	3.8	4.0
Serum globulin, %	3.6	3.1	2.8	2.9	2.1	1.4	1.3	1.5	3.5	2.2	3.0	3.3	2.4	2.8	2.3	2.5	1.3	2.2	2.2	2.3
Cephalin flocculation	+(1)	+(1)	+(1)	+(1)	−ve	−ve	−ve	−ve	+(1)	+(1)	+(1)	+(1)	+(1)	+(1)	+(1)	+(1)	−ve	−ve	−ve	−ve
Serum bilirubin, mg. per 100 ml.	0.2	0.2	0.3	0.2	0.3	0.3	0.2	0.3	0.6	0.4	0.3	0.3	0.2	0.2	0.3	0.2	0.2	0.1	0.2	0.2
Serum alkaline phosphatase, units	11	9	8	6	10	20	18	13	10.5	8.3	7.9	10	17.3	17	16	19	5	9	9.5	9
Blood sugar, mg. per 100 ml.	72	96	101	99	99	79	113	107	53	88	108	110	70	90	120	116	82	90	95	84
Blood urea, mg. per 100 ml.	33	42	30	36	19	20	23	24	47	29	30	33	22	24	25	20	18	23	22	23
Urine																				
albumin	—	—	—	—	+	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—
casts	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
cells	—	—	—	—	+	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—
Erythrocyte sedimentation rate, mm. hr.	20	35	20	36	8	14	13	29	89	79	88	85	30	29	31	32	17	36	31	19
Hemoglobin, G %	13.1	12.7	12.8	12.7	13.5	14.4	13.1	13.1	11.9	12	12.1	11.9	11.9	12.7	13.4	13.5	14.7	14	13.8	14.2
Leukocyte count, cu. mm.	7,700	9,200	6,100	7,900	9,000	10,100	9,200	8,900	5,800	6,200	7,300	8,100	12,200	13,900	11,100	13,600	15,500	11,900	9,900	8,700

Differential count and blood smear were normal in all subjects before and during the trial.

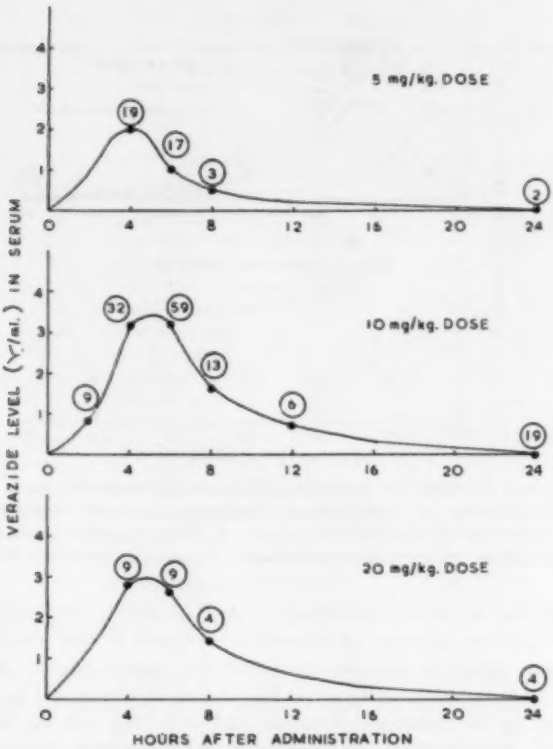

FIG. 2. Mean Verazide serum concentrations in healthy adults following administration of single doses of Verazide in enteric-coated tablets (numbers in the circles refer to number of individuals examined).

and 3 γ per ml., respectively, also at the four-hour period. As it appears that an increase in the dose of more than 10 mg. per kg. of body weight does not raise the serum concentration, this dose probably represents the optimal amount which can be absorbed because of solubility and other factors. At the same time it should be noted that the maximal serum concentration obtained with a single 10 mg. per kg. dose of Verazide is approximately forty times that necessary for tuberculostasis, and that the tuberculostatic concentration is maintained, i.e., >0.1 γ per ml., for at least sixteen to twenty hours. In passing, it might be mentioned that no free isoniazid was detected in any of the sera when enteric-coated tablets were used.

When, however, 10 mg. per kg. of Verazide were given orally in uncoated tablets, the resultant serum curve was different from that seen with the enteric-coated tablets. In figure 3A it may be seen that the peak concentration of Verazide is lower, 2.6 γ per ml., than that found with the enteric-coated tablets

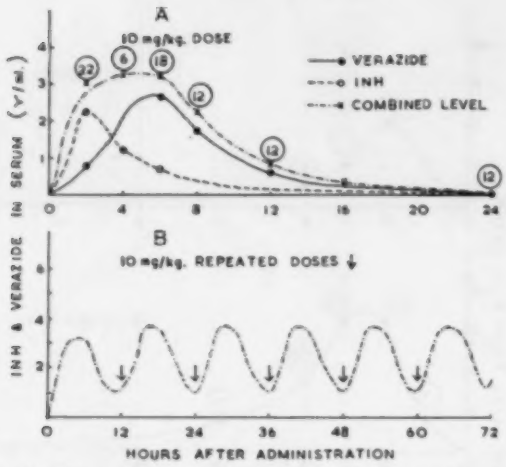

FIG. 3A. Mean Verazide and isoniazid (INH) serum concentrations in healthy adults following administration of single doses of Verazide in uncoated tablets (numbers in the circles refer to number of individuals examined). *B*. Mean combined Verazide and isoniazid serum concentrations following repeated doses of Verazide in uncoated tablets.

(*cf.* figure 2) due to partial hydrolysis of the Verazide to isoniazid. That this is so is shown by the presence of detectable isoniazid in the serum (figure 3A).

From these results it may be calculated that approximately 40 per cent of Verazide is hydrolyzed to isoniazid so that a dose of 10 mg. per kg. is equivalent to 6 mg. per kg. of unchanged Verazide and to 2.4 mg. per kg. of isoniazid. In figure 3*B* the combined curves from figure 3A have been drawn to show the amplitude of concentration of the antituberculous activity when a 10 mg. per kg. dose of uncoated tablets is given every twelve hours. Whether it is preferable to administer the drug in uncoated or enteric-coated tablets will be investigated in the forthcoming clinical trial.

Concentration of Verazide in Cerebrospinal Fluid

Seventeen subjects, patients in the hospital, were given Verazide as enteric-coated tablets in isolated doses of 10 mg. per kg. of body weight. At various times blood and cerebrospinal fluid were collected and the Verazide concentration in each fluid was determined. The individual results are shown in table 4, and the averages at different time intervals are shown in figure 4. It will be noted that, although none of the patients (except *Patient 1*) was suffering from meningeal inflammation at the time of examination, the serum and cerebrospinal fluid concentrations were similar. The close correspondence of the mean serum and cerebrospinal fluid concentrations in the "normal" subject is shown in figure 4. On these findings it can justifiably be assumed that the concentration of Verazide in the cerebrospinal fluid will be equal to, but more

TABLE 4

SERUM AND CEREBROSPINAL FLUID CONCENTRATIONS AFTER ADMINISTRATION OF VERAZIDE 10 Mg. per Kg. of Body Weight

Patient	Age	Sex	Condition	Verazide Concentration (γ per ml.)		Time After Dose
				Serum	C.S.F.*	
						hours
1	5	M	Tuberculous meningitis	4	10	4
2	4	F	Post-tuberculous meningitis	3	5	4
3	60	F	Spinal tumor	4	4	4
4	28	M	Post-tuberculous meningitis	4	4	4
5	42	M	Glioblastoma multiforme	2	2	4
6	48	M	Fractured skull	3	2	4
7	26	F	Spinal tumor	3	2	4
8	42	M	Spinal tumor	3	4	5
9	65	M	Cerebral aneurysm	2	2	5
10	70	M	Carcinoma of lung	4	3	5
11	50	M	Cerebral atrophy	3	4	6
12	68	M	Epilepsy	3	4	6
13	32	M	Post-tuberculous meningitis	3	3	6
14	5	M	Post-meningococcal meningitis	6	5	6
15	46	M	Epilepsy	1	1.5	8
16	35	M	Concussion	4	3	8
17	42	M	Glioblastoma multiforme	0	0	24

*CSF—Cerebrospinal fluid

likely greater than, that of the blood in cases of tuberculous meningitis. This is suggested by the result in *Patient 1*, the only case available to us.

Concentration of Verazide in Urine

A preliminary attempt was made to detect Verazide and any of its likely degradation products, e.g., isoniazid, isonicotinic acid, acetyl isoniazid, in the urine. One subject was studied in this experiment who, on previous tests, showed high serum concentrations of the drug. Examinations of the urine at four, eight, twelve, and twenty-four hours after oral administration of Verazide failed to

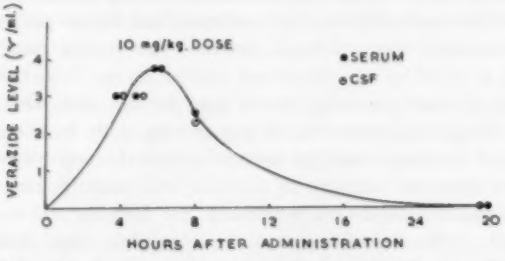

FIG. 4. Mean Verazide serum and cerebrospinal fluid (CSF) concentrations following administration of single doses of Verazide in enteric-coated tablets.

show the presence of the drug or related derivatives in the urine. At this stage it is not possible to draw any conclusions on the form in which Verazide is excreted in the urine, and further work is obviously advisable.

Discussion

The aspects of the pharmacology of Verazide which have been investigated here are those of special interest from the clinical point of view. A number of interesting points have emerged.

In an earlier publication (1) Verazide was shown to be markedly effective in guinea pig tuberculosis when given intramuscularly as a depot dose of 20 mg. per kg. of body weight at weekly intervals. It was hoped that a similar method of treatment might be evolved for the human subject. However, the findings described here showed that the depot-dose concept was quite impracticable for two reasons. First, concentrated aqueous suspensions required for human use form a thick gel which is impossible to inject. Second, if a free-flowing suspension is made with ethyl oleate as a suspending fluid, the degree of local tissue damage is so great that an aseptic abscess results. Thus, although Verazide is admirably suited for depot dosing by virtue of its low solubility, it is quite unsuitable, as are other low solubility hydrazones, because of the local tissue damage. Accordingly, Verazide can be given only by mouth.

The oral administration of Verazide raises two questions, both of which have been thoroughly investigated. The first deals with its stability in gastric juice (table 1) and the second with the amount absorbed from the small intestine. With regard to stability, it was shown (figure 3*A*) that Verazide can be easily protected from acid hydrolysis by giving the drug in enteric-coated tablets. On the other hand, when given as uncoated tablets, approximately 40 per cent is hydrolyzed in the gastric juice, the products of the hydrolysis being isoniazid and veratric aldehyde. The total antituberculous activity of serum in the latter case (figure 3*B*) is higher than that reached by using the enteric-coated tablets (figure 2). It appears that Verazide could be given more easily in the uncoated tablet form but, as the chronic toxicity in humans was determined with enteric-coated tablets, the former cannot be recommended until the effect of hydrolysis has been investigated clinically.

The oral dosage of Verazide was determined using enteric- and nonenteric-coated tablets. The results (figures 2, 3) indicate that 10 mg. per kg. are advisable as a single dose since lower dosages produce low serum concentrations, but higher dosages, at least for enteric-coated tablets, do not raise the concentration significantly above that given by the 10 mg. per kg. dose. In view of the low toxicity of the drug, it appears that 20 mg. per kg. daily in two doses of 10 mg. per kg. night and morning would be optimal initial therapy with this drug.

In regard to systemic toxicity in animals and man, it can be confidently stated that Verazide exhibits an extremely low toxicity when compared with isoniazid for all species tested, e.g., mice, guinea pigs, dogs, rabbits, and man. Of especial interest is the chronic toxicity in humans. In spite of repeated tests in clinically advanced cases, no evidence of physiologic or biochemical damage

could be detected (table 3). These findings show that Verazide can be given in enteric-coated tablets in doses of 20 mg. per kg. of body weight daily without ill effect. It might also be noted that partial hydrolysis of Verazide will not increase its toxicity, since veratric aldehyde has the same LD_{50} as Verazide in contrast to Salizid and vanizide (or Phtivazid), the corresponding aldehydes of which are far more toxic than the parent hydrazones (table 2). One final point which might also be added here is that Verazide will not cause a peripheral neuritis in man because chemically it cannot react with pyridoxal.

It was possible to show that the Verazide concentration in the cerebrospinal fluid was equal to, and in some cases higher than, that found in the serum. It might be mentioned that the patients used for these determinations were not suffering from any measurable meningeal inflammation. Thus, it is most possible that in tuberculous meningitis the concentration of Verazide in the cerebrospinal fluid would be higher than in serum.

Finally, a few preliminary tests were made in an attempt to determine the form in which the drug was excreted in the urine. So far these efforts have been unrewarding. No Verazide or any of its likely breakdown products were detected chemically. One point on which it is possible to be fairly certain in the absence of experimental proof is that Verazide cannot be inactivated by acetylation due to its hydrazone structure.

In conclusion, all of the evidence so far adduced points to Verazide as having a more favorable chemotherapeutic index than isoniazid for the treatment of human tuberculosis.

Summary

Some aspects of the properties and pharmacology of Verazide, 3:4-dimethoxybenzal isonicotinyl hydrazone, have been described. Although at pH below 2.4 the drug in solution is rapidly hydrolyzed to isoniazid and veratric aldehyde, 60 per cent remains unchanged when given to humans as compressed uncoated tablets.

The serum and cerebrospinal fluid concentrations have been measured following oral administration, and it is concluded that the optimal daily dose in humans is 20 mg. per kg. of body weight given as two equal doses of 10 mg. per kg. twice daily. The serum concentrations with this regimen fluctuated between ten and forty times the minimal concentration required to inhibit *Mycobacterium tuberculosis*.

Toxicity tests in animals and a prolonged chronic toxicity trial in humans showed that the dose recommended is quite safe. Clinical, biochemical, and hematologic observations failed to reveal any evidence of acute or chronic toxicity in human subjects.

Because of its low solubility it would appear that Verazide would be well suited for intermittent injection, as has been demonstrated in animals. However, the acute local toxicity of concentrated suspensions contraindicates its use in this manner, and intravenous injection carries the danger of embolic complications.

Verazide is regarded as potentially superior to isoniazid for the treatment of human tuberculosis since higher concentrations can be maintained *in vivo* without producing any toxic reactions. A clinical trial of this compound in the treatment of tuberculosis has now been commenced and will be reported on later.

Sumario

Quimioterapia de la Tuberculosis: II. Algunas Observaciones sobre la Farmacología de la Veracida

Descríbense aquí algunas fases de las propiedaes y la farmacología de la veracida, 3:4-dimetoxibenzal-isonicotinil-hidrazona. Aunque a un pH inferior a 2.4, la droga disuelta se hidroliza rápidamente en isoniacida y aldehido verátrico, un 60 por ciento permanece inalterado cuando se administra a seres humanos en forma de comprimidos sin revestimiento.

Midiéronse las concentraciones en el suero y el líquido céfalorraquídeo a continuación de la administración oral, deduciéndose que la dosis diaria óptima en el hombre es de 20 mg. por kg. de peso, administrado en dos dosis iguales de 10 mg. por kg. de peso dos veces diarias. Con este régimen, las concentraciones séricas superaron entre diez y cuarenta veces la concentración mínima requerida para inhibir el *Mycobacterium tuberculosis*.

Ensayos de la toxicidad en los animales y una prolongada prueba de la toxicidad crónica en el hombre demostraron que la dosis recomendada es bastante inocua. Datos clínicos, bioquímicos y hematológicos no revelaron signo alguno de toxicidad aguda o crónica en seres humanos.

Debido a su poca solubilidad, parece que la veratrina se prestaría para inyección intermitente, según se ha demostado en los animales. No obstante, la toxicidad aguda local de las suspensiones concentradas contraindica su empleo en esta forma, y la inyección intravenosa entraña el riesgo de complicaciones embólicas.

Se considera la veracida como potencialmente superior a la isoniacida para el tratamiento de la tuberculosis humana, dado que cabe mantener concentraciones más altas *in vivo* sin producir reacciones tóxicas. Ya se ha comenzado una prueba clínica que se describirá después.

Resume

Chimiothérapie de la tuberculose: II. Quelques observations sur la pharmacologie de al Verazide

Certains aspects des propriétés et de la pharmacologie de la verazide, 3:4-diméthoxybenzol isonicotinyl hydrazone, ont été décrits. Alors qu'à un pH inférieur à 2,4 la drogue en solution est rapidement hydrolysée en isoniazide et en aldéhyde vératrique, 60% demeurent inchangés quand la drogue est administrée à l'homme sous forme de comprimés non enrobés.

Les concentrations du sérum et du liquide céphalo-rachidien ont été déterminés après l'administration orale et il est admis que la dose quotidienne optima chez l'homme est de 20 mg par kg. de poids corporel administrée en deux doses égales de 10 mg par kg. deux fois par jour.

Les concentrations sériques obtenues par cette posologie fluctuaient entre dix et quarante fois la concentration minima requise pour inhiber le *Mycobacterium tuberculosis*.

Les tests de toxicité chez l'animal et les preuves de la toxicité chronique prolongée chez l'homme montraient que la dose préconisée est absolumment sûre. Les données cliniques, biochimiques et hématologiques n'ont révélé aucune signe de toxicité aiguë ou chronique.

En raison de solubilité faible, il semblerait que la vérazide convienne l'injection intermittente, comme il a été démontré chez l'animal. Toutefois, la toxicité aiguë locale des suspensions concentrées contre-indique ce mode d'emploi, et l'injection intra-veineuse entraîne le danger de complications emboliques.

La vérazide est considérée comme virtuellement supérieure a l'isoniazide pour le traitement de la tuberculose humaine car des concentrations plus élevées peuvent être maintenues *in vivo* sans aucune réaction de toxicité. Une expérimentation clinique est en cours et sera raportée ulterieur.

Acknowledgments

The writers wish to thank the National Health & Medical Research Council, the contributors to the Argus Research Fund, and Dr. A. E. Rowden White, C.M.G., for financial assistance; Miss Janice Edgar for assistance in the animal experiments; and the staff, students, patients, and clinicians for their willing cooperation. Also, they wish to thank Dr. J. Cymerman-Craig for supervising the initial stage of the estimation of Verazide, and the Glaxo Laboratories of Australia for the supply of Verazide used in this study.

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U. S. VETERANS ADMINISTRATION—ARMED FORCES COOPERATIVE STUDIES OF TUBERCULOSIS

VI. Survival Among Patients with Miliary and Meningeal Tuberculosis (1948–1955)[1]

JOHN H. WILLIAMS, JR.[2]

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Introduction

Coincident with the study of the antimicrobial therapy of pulmonary tuberculosis made by the Veterans Administration, Army, and Navy (1–4), data have been collected on 772 patients treated for miliary or meningeal tuberculosis. These patients were treated with a variety of dosages of different antimicrobial agents, but for purposes of comparison the cases were classified into four treatment groups. The treatment regimens were not assigned by random allocation, but were determined by the advent of additional antimicrobial agents.

The earliest patients included in this study were treated with streptomycin or dihydrostreptomycin alone, ordinarily for short duration (120 days), and these patients constituted the first treatment group. No distinction between streptomycin and dihydrostreptomycin was made in this study. The second group consisted of patients treated during 1947 and 1948 with streptomycin-sulfone (Promin®). The third group consisted of patients treated with streptomycin-para-aminosalicylic acid (PAS), and the last group consisted of patients treated since 1952 with either isoniazid-streptomycin-PAS or isoniazid-streptomycin. Almost all of this latter group were treated with three antimicrobial agents, although for convenience this treatment group will be referred to as the isoniazid-streptomycin group.

The dosages and duration of administration of the drugs varied both within and among the treatment groups. Streptomycin was given either intramuscularly or intrathecally in dosages of 1, 2, or 3 gm., ordinarily daily in the initial stage of treatment, with a subsequent change to twice-a-week administration. Intrathecal administration, once common, has rarely been used since the advent of isoniazid therapy. Para-aminosalicylic acid was normally given in daily doses of 12 gm. and isoniazid was given daily in three doses of 100 mg. There was a radical change in the duration of antimicrobial therapy following the introduction of PAS, and patients treated with streptomycin in combination with either PAS, isoniazid, or both, received antimicrobial therapy of longer duration than patients treated with earlier regimens.

Materials and Methods

The primary purpose of the present study was to determine the survival rate among patients with miliary and meningeal tuberculosis treated with different antimicrobial

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[1] Prepared at the request of the Executive Committee of the Conference on Chemotherapy of Tuberculosis of the Veterans Administration, Army, and Navy.

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agents. To accomplish this end, the cases were classified into the different treatment groups according to the initial therapy received. Patients initially treated with streptomycin-PAS who later received isoniazid were classified as the streptomycin-PAS group. While this procedure resulted in an overstatement of the survival rate among treatment groups prior to the one in current usage, the bias was not considered serious since the number of such cases was few and most of the changes in therapy occurred late in treatment when the likelihood of death had diminished.

The survival rates found in this study refer only to treated patients. The data were all collected from hospitals with a tuberculosis service where a delay in diagnosis was less probable than in some other situations. It is likely, therefore, that the survival rates presented here are higher than might ordinarily be encountered.

An initial report on patients in the study was received at the start of treatment and follow-up reports were submitted each November. Although many patients were lost to follow-up observation by the treating hospital following the patient's discharge, it was possible through Veterans Administration records to determine the survival status of all patients. Thus, all surviving patients treated with streptomycin alone or with streptomycin-sulfone have been followed for five years; all patients treated with streptomycin-PAS were followed for at least three years. Since isoniazid-streptomycin was given to patients who started their therapy as late as 1955, the period of follow-up observation on these patients varied. The survival rate for this group was an estimate, using actuarial methods, based on the observations of those patients who had been observed for longer periods. The survival rates for the fourth and fifth years for patients treated with streptomycin-PAS were computed in the same manner.

Only patients who received their initial chemotherapy for miliary or meningeal tuberculosis at a hospital participating in the cooperative study were included in the calculated survival data. This requirement has been relaxed slightly for cases of miliary tuberculosis without meningitis. Cases of this type have been included for the past two years if the patients were admitted to a study hospital within two weeks of the start of treatment. Because of the low mortality rate for this group, the inclusion of such cases should not seriously bias the data.

The criteria for inclusion specified that only proved cases of tuberculosis were to be reported. It was not possible to check every individual case, especially those patients treated prior to 1952, to verify the fact that tubercle bacilli were demonstrated bacteriologically. However, it was possible to re-examine the reports of patients treated with isoniazid-streptomycin, and for these patients the reporting hospitals adhered to the criteria.

Not in all patients with tuberculous meningitis were tubercle bacilli recovered from the cerebrospinal fluid. Of the 74 patients with tuberculous meningitis treated with isoniazid-streptomycin, in only 39 (53 per cent) were tubercle bacilli demonstrated in the cerebrospinal fluid. Among the remaining 35 patients, tubercle bacilli were isolated from the sputum, gastric washings, urine, et cetera, but not from the cerebrospinal fluid. Among these 35 cases, nine deaths occurred as compared with six among the 39 patients with bacteriologically positive cerebrospinal fluid findings.

The patients were all adults and almost all (more than 99 per cent) were males. Approximately one-half of the patients were whites; approximately 60 per cent were less than forty years of age. There were slight differences in the race and age compositions among the various treatment groups (table 1).

Slightly fewer whites and more patients more than sixty years old were treated with isoniazid-streptomycin than were treated with the earlier drug regimens.

Results

Miliary tuberculosis: One of the more dramatic results from the introduction of effective combined chemotherapy has been the decline in the incidence of meningitis among patients with miliary tuberculosis (tables 2 and 3).

TABLE 1

AGE AND RACE OF MILIARY AND MENINGEAL CASES OF TUBERCULOSIS BY TYPE OF TUBERCULOSIS AND REGIMEN

Type of Tuberculosis and Regimen	Race					Age at Start of Therapy						
	Total Cases	Percentage of Total Cases of Indicated Race				Total Cases	Percentage of Total Cases of Indicated Age					
		White	Negro	Other	Unknown		Less than 30	30–39	40–49	50–59	60+	Unknown
1. Miliary only												
Streptomycin only	65	55	45	0	0	65	40	18	17	17	8	0
Streptomycin-sulfone	14	50	43	7	0	14	21	43	0	29	7	0
Streptomycin-PAS	89	58	36	6	0	89	25	25	15	26	8	1
Isoniazid-streptomycin	87	41	53	6	0	87	25	28	16	12	17	2
2. Miliary followed by meningitis*												
Streptomycin only	35	43	57	0	0	35	51	20	14	12	3	0
Streptomycin-sulfone	16	25	69	6	0	16	44	38	6	6	6	0
Streptomycin-PAS	22	68	32	0	0	22	23	32	14	22	9	0
Isoniazid-streptomycin	0	—	—	—	—	0	—	—	—	—	—	—
3. Meningitis only												
Streptomycin only	122	66	31	3	0	122	40	31	6	21	2	0
Streptomycin-sulfone	39	62	38	0	0	39	31	31	3	33	2	0
Streptomycin-PAS	88	56	42	2	0	88	30	35	17	16	2	0
Isoniazid-streptomycin	74	45	53	2	0	74	20	51	11	12	6	0
4. Miliary and meningitis												
Streptomycin only	50	52	44	2	2	50	36	28	14	20	2	0
Streptomycin-sulfone	13	46	46	8	0	13	47	15	15	23	0	0
Streptomycin-PAS	30	53	43	4	0	30	13	37	17	27	0	6
Isoniazid-streptomycin	28	54	43	3	0	28	32	18	29	14	7	0

* Includes patients with miliary tuberculosis who developed meningitis following the start of therapy for miliary tuberculosis alone.

Among 100 patients with miliary tuberculosis, who were treated with streptomycin alone, 35 developed meningitis within a year of the start of therapy for miliary tuberculosis. If those patients who died from miliary tuberculosis without the development of meningitis are considered as losses to observation, the incidence rate of meningitis was 40 per cent. Among the 30 patients treated with streptomycin-sulfone, the rate was 60 per cent. Those patients treated for miliary tuberculosis with streptomycin-PAS had a much lower rate (22 per cent), and among the 87 patients treated with isoniazid-streptomycin, 44 of whom were observed for a year or longer, none developed meningitis. This latter observation is in marked contrast to previous experience, and would seem to indicate that the combination of isoniazid-streptomycin was effective in lowering the incidence of tuberculous meningeal complications among patients with miliary tuberculosis even if, as has been reported (5), it cannot completely prevent it.

The percentage of patients with miliary tuberculosis uncomplicated by menin-

TABLE 2

NUMBER OF PATIENTS WITH MILIARY TUBERCULOSIS WHO DEVELOPED MENINGITIS AFTER THE START OF THERAPY FOR MILIARY TUBERCULOSIS BY DURATION OF OBSERVATION AND REGIMEN

Duration of Observation After Start of Therapy	Streptomycin Only		Streptomycin-Sulfone		Streptomycin-PAS		Isoniazid-Streptomycin	
	Miliary Cases at the Start of Period	Cases of Meningitis During Period	Miliary Cases at the Start of Period	Cases of Meningitis During Period	Miliary Cases at the Start of Period	Cases of Meningitis During Period	Miliary Cases at the Start of Period	Cases of Meningitis During Period
Observed during								
First month	100	4	30	4	111	1	87	0
Second month	90	7	24	3	106	2	80	0
Third month	83	3	20	6	103	1	75	0
Fourth month	78	6	14	1	101	5	74	0
Fifth month	66	8	12	1	91	3	69	0
Sixth month	55	5	11	0	86	2	64	0
Seventh month	48	1	11	1	83	2	60	0
Eighth month	44	0	10	0	81	0	54	0
Ninth month	43	1	10	0	81	1	50	0
Tenth month	42	0	10	0	77	4	48	0
Eleventh month	40	0	10	0	72	0	45	0
Twelfth month	39	0	10	0	72	1	44	0

TABLE 3

ESTIMATED PERCENTAGE OF PATIENTS WITH MILIARY TUBERCULOSIS WHO DEVELOPED MENINGITIS BY END OF PERIOD*

Duration of Observation After Start of Therapy	Streptomycin Only	Streptomycin-Sulfone	Streptomycin-PAS	Isoniazid-Streptomycin
At end of				
First month	4	14	1	0
Second month	12	25	3	0
Third month	15	48	4	0
Fourth month	22	51	8	0
Fifth month	31	56	11	0
Sixth month	38	56	14	0
Seventh month	39	60	16	0
Eighth month	39	60	16	0
Ninth month	40	60	17	0
Tenth month	40	60	21	0
Eleventh month	40	60	21	0
Twelfth month	40	60	22	0

* Computed by use of life-table procedures. Deaths from miliary tuberculosis uncomplicated by meningitis were considered losses to observation.

gitis who survived for the indicated period following the start of therapy is shown in figure 1. All patients who subsequently developed meningitis were excluded from these calculations and their experience was calculated separately. The actual two-year survival rate for patients treated with streptomycin only was

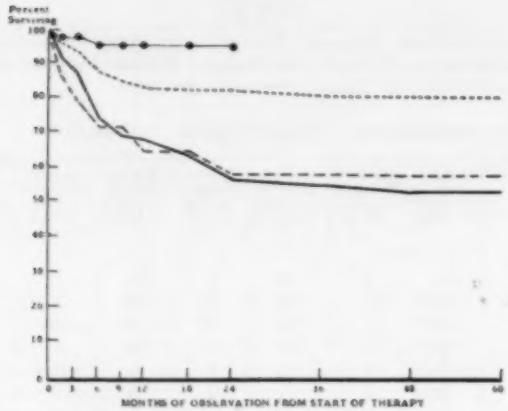

FIG. 1. Survivorship curve—miliary tuberculosis only
●-●-●- isoniazid-streptomycin
– – – – – streptomycin-PAS
— — — — streptomycin-sulfone
————streptomycin only
Isoniazid-streptomycin—87 cases
Streptomycin-PAS—89 cases
Streptomycin-sulfone—14 cases
Streptomycin only—65 cases

56 per cent; with streptomycin and sulfone, 57 per cent; and with streptomycin-PAS, 81 per cent. The estimated survival rate for patients treated with isoniazid-streptomycin was 95 per cent, 14 per cent higher than was found among patients treated with streptomycin-PAS.

The five-year survival rate for patients with miliary tuberculosis without meningitis treated with streptomycin only was 52 per cent; with streptomycin-sulfone, 57 per cent; and with streptomycin-PAS, 80 per cent.

In figure 2 may be seen the survival rate among patients with miliary tuberculosis who developed meningitis after the start of chemotherapy for their miliary disease. The survival rates were measured from the onset of the meningitis. Only one of 35 patients treated with streptomycin alone and one of 16 patients treated with streptomycin-sulfone survived five years. Currently, 8 of 22 patients with miliary tuberculosis treated with streptomycin-PAS who subsequently developed meningitis are still alive.

Tuberculous meningitis (figure 3): The two-year survival rate for 74 patients with tuberculous meningitis without miliary tuberculosis treated with isoniazid-streptomycin was 80 per cent as compared with a rate of 51 per cent for 88 patients who received streptomycin-PAS; 21 per cent of 122 patients treated with streptomycin only; and 15 per cent of the 39 patients with tuberculous meningitis treated with streptomycin-sulfone.

At five years, 19 per cent of the patients with tuberculous meningitis treated with streptomycin alone were still alive, as were 15 per cent of the patients treated

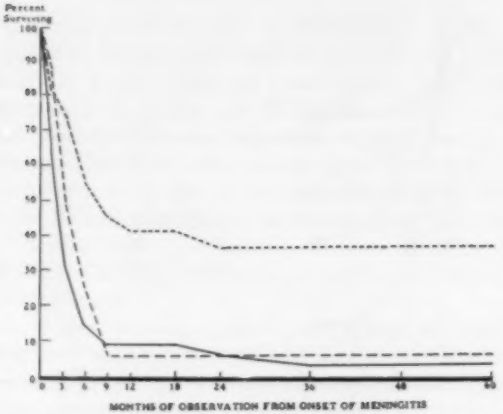

FIG. 2. Survivorship curve—miliary tuberculosis followed by meningitis

- - - - - streptomycin-PAS
— — — — streptomycin-sulfone
———— streptomycin only

Streptomycin-PAS—22 cases
Streptomycin-sulfone—16 cases
Streptomycin only—35 cases

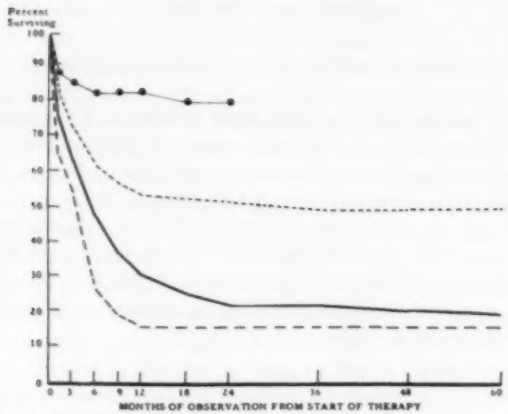

FIG. 3. Survivorship curve—meningitis only

●-●-●- isoniazid-streptomycin
- - - - - streptomycin-PAS
———— streptomycin only
— — — — streptomycin-sulfone

Isoniazid-streptomycin—74 cases
Streptomycin-PAS—88 cases
Streptomycin-sulfone—39 cases
Streptomycin only—122 cases

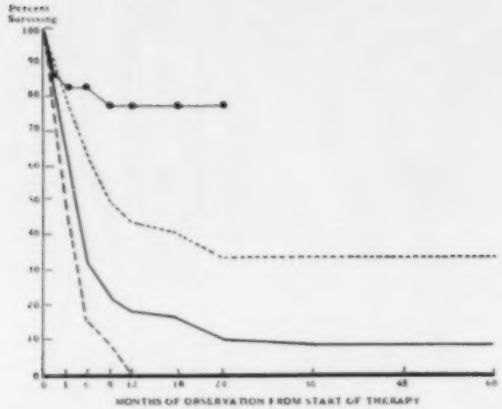

FIG. 4. Survivorship curve—miliary tuberculosis and meningitis

●—●—● isoniazid-streptomycin
– – – – – streptomycin-PAS
———— streptomycin only
— — — — streptomycin-sulfone

Isoniazid-streptomycin—28 cases
Streptomycin-PAS—30 cases
Streptomycin-sulfone—13 cases
Streptomycin only—50 cases

with streptomycin-sulfone, and 50 per cent of those who received streptomycin-PAS.

The survival rate for patients with both miliary and meningeal tuberculosis at the start of chemotherapy is shown in figure 4. At two years, 77 per cent of the 28 patients treated with isoniazid-streptomycin had survived as compared with 33 per cent of those treated with streptomycin-PAS, 0 per cent for streptomycin-sulfone, and 10 per cent of those treated with streptomycin only.

Five years after the start of treatment the survival rate for streptomycin alone was 8 per cent (4 of 50 cases). None of the 13 patients treated with streptomycin-sulfone survived, and 10 of the 30 patients treated with streptomycin-PAS were still reported as living.

DISCUSSION

Despite the fact that this was not a study of regimens assigned by random allocation, it seems obvious that the regimens of isoniazid-streptomycin or isoniazid-PAS were superior to the earlier regimens. Indeed the death rates among patients with miliary tuberculosis, meningeal tuberculosis, or with a combination of miliary and meningeal tuberculosis were significantly lowered coincident with the addition of isoniazid to the treatment.

It is possible that the characteristics of the patients starting treatment had changed during the study period, but it is highly improbable that these changes

would coincide with changes in the type of antimicrobial therapy, especially as there does not appear to be any such trend within treatment groups. For example, 15 per cent of the patients starting treatment for tuberculous meningitis with isoniazid-streptomycin during 1952–1953 died within ninety days of the start of treatment. Exactly the same percentage of patients who started treatment during 1954 and 1955 died within ninety days. Nor can the differences be accounted for by differences in race or age since the patients treated with isoniazid-streptomycin did not differ greatly in these respects from patients treated with other drug combinations. Likewise, the duration of therapy, while of importance, cannot account for the differences among the treatment groups during the early months of therapy.

An examination of deaths among patients with miliary or meningeal tuberculosis revealed that race was not an important factor. Age was important. For each meningitic treatment group, the death rate for patients between forty and fifty-nine years was approximately 10 per cent higher than for patients less than forty years of age. Among 9 patients with meningitis who were sixty or older when treatment started, 5 died within a month of the start of therapy, and 2 died between one and two years following the initiation of therapy. Two patients, both treated with isoniazid-streptomycin-PAS, are still living. One has been observed for more than three years and the other for slightly more than one year.

It was extremely difficult to distinguish the effect of duration of chemotherapy from the effect of regimen, since the two are highly correlated. In general, the patients who were treated subsequent to the introduction of PAS into the treatment of tuberculosis received chemotherapy of long duration (in excess of one year). Those patients treated with either streptomycin alone or in combination with sulfone ordinarily received an initial four-month course, but many then received additional drug therapy either with the same drugs or with newer drugs as they became available. Since the institution of additional drug therapy was often the result of a poor prognosis or of a suspected relapse, a comparison of patients who received a four-month course with patients who received more chemotherapy was not possible.

There were some indications of the non-permanent nature of the efficacy of antimicrobial therapy of short duration for meningeal tuberculosis, as evidenced by a number of relapses within a month of the termination of four-month courses of streptomycin. There was also an increase in the likelihood of death among patients who left the hospital against medical advice before a year of treatment. While not enough data were available to determine the optimal duration of treatment—if indeed one existed—it was apparent that the earlier in the course of treatment a patient left the hospital, the greater the likelihood of death. Among those patients with tuberculous meningitis treated with streptomycin only or streptomycin-sulfone, 7 left before receiving eight months of treatment. Four of these patients died within two months, and one died eight months after leaving the hospital.

The only patients who received less than a year of therapy with isoniazid-

streptomycin or streptomycin-PAS were 10 patients who left the hospital against medical advice. Four of the 10 patients died within a year of discharge, 2 in each treatment group. This was a higher rate than was found among patients continuing treatment even though there was no reason to believe at the time of discharge that those patients who left against medical advice constituted a high-risk group.

Summary

The survival rate for 772 patients with miliary, meningeal, or miliary and meningeal tuberculosis treated in hospitals participating in the Veterans Administration-Armed Forces Cooperative Study of the Chemotherapy of Tuberculosis was determined for different types of antimicrobial agents, including isoniazid-streptomycin, most often with PAS in addition. Since the introduction of isoniazid in 1952, the estimated survival rate at two years after the start of therapy for miliary tuberculosis was 95 per cent; for meningeal tuberculosis, 80 per cent; and for miliary and meningeal tuberculosis, 77 per cent.

The addition of isoniazid to the therapy of miliary tuberculosis has been effective in lowering the incidence of meningitis among patients with miliary tuberculosis.

Increasing age decreased the probability of survival for miliary and meningitic patients.

The incidence of miliary and of meningeal tuberculosis was higher among non-whites than among whites. This was evidenced by the fact that, while non-whites constitute approximately one-tenth of the veteran population, nearly one-half of the patients reported in the present study were non-white veterans. However, race was not an important consideration in the survival of treated patients.

Premature or irregular discharge resulted in an increase in the probability of death among patients with tuberculous meningitis.

Sumario

La Sobrevivencia entre los Enfermos con Tuberculosis Miliar (Granulia, y Meníngea: Informe del Estudio Cooperativo Ejecutado por la Administración de Veteranos, el Ejército y la Armada, 1948 a 1955

La tasa de sobrevivencia para 772 enfermos que tenían tuberculosis miliar (granulia), meníngea o miliar y meníngea y que fueron tratados en hospitales que participaban en el Estudio Cooperativo de la Quimioterapia de la Tuberculosis Ejecutado por la Administración de Veteranos, el Ejército y la Armada, fué determinada para distintas clases de medicamentos antimicrobianos, comprendiendo estreptomicina e isoniacida, más a menudo con PAS. Desde la introducción de este régimen en 1952, la tasa de sobrevivencia calculada a los dos años de la iniciación de la terapéutica fué para la granulia de 95 por ciento; para la tuberculosis meníngea, 80 por ciento; y para la tuberculosis miliar y meníngea, 77 por ciento.

La adición de isoniacida a la terapéutica de la granulia ha logrado rebajar la incidencia de la meningitis entre los enfermos de granulia.

Una edad mayor rebajó las probabilidades de sobrevivencia para los enfermos con granulia y meningitis.

La incidencia de granulia y de tuberculosis meníngea fué mayor entre los sujetos que no eran de raza blanca que entre los blancos, según evidencia el hecho de que, mientras

que los primeros constituyen aproximadamente la décima parte de la población de veteranos, casi la mitad de los enfermos discutidos en esta comunicación no era de raza blanca. Sin embargo, la raza no representó un factor importante en la sobrevivencia de los enfermos tratados.

Las altas prematuras o irregulares dieron por resultado un aumento en las posibilidades de muerte entre los enfermos de meningitis tuberculosa.

Resume

La survie parmi les malades atteints de tuberculose miliaire et méningée: Rapport de l'étude faite en collaboration par Veterans Administration, Army et Navy de 1948 à 1955

Le taux de survie pour 772 malades affectés de tuberculose miliaire, méningée, ou miliaire et méningée traités dans les hôpitaux participant à l'étude de la chimiothérapie de la tuberculose comprenant ceux de *Veterans Administration Army* et *Navy*, a été déterminé pour différents types d'agents antibactériens comprenant la streptomycine et l'isoniazide, le plus souvent associées au PAS. Depuis l'introduction de cette thérapeutique en 1952, 2 ans après le début du traitement le taux de survie était selon les estimations: pour la tuberculose miliaire 95%; pour la tuberculose méningée, 80%; et pour la tuberculose miliaire et méningée, 77 pour cent.

L'adjonction d'isoniazide au traitement de la tuberculose miliaire s'est montrée efficace, abaissant l'incidence de la méningite parmi les malades attents de tuberculose miliaire.

La taux de survie probable s'abaisse pour les malades atteints de tuberculose miliaire et méningée à mesure que l'âge augmente.

L'incidence de la tuberculose miliaire et de la tuberculose méningée était plus élevée parmi ceux qui n'étaient pas de race blanche que parmi les blancs comme le démontrait le fait que, tandis que les non-Blancs constituent approximativement un dixième de la population des anciens combattants, à peu près la moitié des malades compris dans cette étude ètaient des anciens combattants n'appartenant pas à la race blanche. Toutefois, la race ne jouait pas un role important dans la survie chez les malades traités.

La sortie irrégulière ou prématurée de l'hôpital produisait une augmentation dans le taux de probabilité du décès parmi les malades atteints de méningite tuberculeuse.

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PATHOGENESIS AND TREATMENT OF PULMONARY TENSION CAVITIES[1]

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Introduction

The concept of the tension cavity is relatively recent. Vineberg and Kunstler (1), in their instructive paper, reviewed the literature and named those who introduced the idea that mechanical factors could be responsible for the pathogenesis of many of the cavities seen clinically in pulmonary tuberculosis.

Vineberg and Kunstler credit Hall (2) with first advancing the idea that a blocked bronchus could cause ballooning out of a cavity. In 1928, Reinders (3) advanced the idea that the volume of a cavity was not actually due to destroyed lung tissue, but rather to collapsed lung parenchyma that was still capable of re-expansion. Pearson (4), in 1930, measured the pressure in a large cavity by transthoracic needling and found the pressure to be supra-atmospheric. Pearson concluded that many large cavities were due to such inflation. In 1931, Brooke (5) needled a large cavity, found supra-atmospheric pressures, and concluded that he was dealing with a valvelike bronchial obstructive mechanism. Korol (6), in 1935, reported a case of rapid enlargement of a cavity following partial occlusion of the draining bronchus by a blood clot, with prompt return of the cavity to its previous size after discharge of the clot. Korol stated:

> The cause of the sudden change in the size of a tuberculous cavity is to be looked for in the state of the draining bronchus. A temporary narrowing of the bronchial channel, as by a blood clot, catarrhal swelling, caseous material, etc., may cause a ball-valve mechanism with respiratory obstruction and rapid enlargement of the cavity. Relief of the obstruction leads to contraction of the cavity.

In 1937, Elocesser (7) applied the term "blocked cavities" and stated that "the relation of pulmonary cavities to the bronchial tree is important, not only theoretically, but clinically because a partially or intermittently blocked bronchus can keep a cavity inflated and prevent its cure."

Coryllos (8), in 1936, published a most instructive paper on the tension cavity. He stated that following excavation of a caseous area, a hole remains in the lung, communicating with the outside world by one or more bronchial outlets. The fate of the hole depends on the behavior of the lesions in the draining bronchus which presents marked lesions early in the disease. He wrote:

> Three eventualities may occur: (*1*) the bronchial outlets of a cavity may remain open; (*2*) they may close; or (*3*) they may become narrowed. In the last case a one-way valve mechanism may develop, as in foreign bodies or tumors of the bronchi, which allow ingress of air during inspiratory expansion of the bronchi, whereas during expiratory collapse of the same bronchi obstruction becomes complete and egress of air is prevented.

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[1] From the Tuberculosis Service, Veterans Administration Hospital, Oteen, North Carolina.

Thus he describes the mechanism of cavity formation. By transthoracic needling he demonstrated that cavities with an open bronchus had atmospheric pressures, high oxygen content (16 to 19 per cent), and low carbon dioxide content (1 per cent or less); and that cavities with check-valve mechanism had supra-atmospheric pressures, reduced oxygen content (10 to 17 per cent), and increased carbon dioxide content (2 to 3 per cent).

Vineberg and Kuntsler (1) needled 67 cavities and found that 42 per cent were of the tension variety. They attribute failure of cavity closure after thoracoplasty largely to the fact that an unrecognized tension cavity was present before operation. They strongly recommend needling large cavities to determine whether they are of the tension or non-tension variety.

The purpose of the present report is to emphasize the frequency of tension cavities in clinical tuberculosis and to stress the role of endobronchial obstruction in the causation of such cavities. This obstruction may be due to organic pathologic changes in the bronchial channel, mechanical factors, or a combination of both. Organic changes causing obstruction may be endobronchial infiltrations encroaching on the lumen, mucosal swelling, granulomatous lesions, and so forth. Mechanical factors causing bronchial obstruction may be angulation of the bronchus resulting from anatomical configuration; kinking of the bronchus by external pressure as may be encountered in collapse therapy (pneumothorax, pneumoperitoneum, phreniclasia, thoracoplasty); temporary blockage by inspissated secretions, blood clots, debris, and other mechanisms.

If there is conviction that endobronchial obstruction is the fundamental cause of tension cavity formation, then it is obvious that therapy must be directed primarily toward relieving the obstruction. The new chemotherapeutic agents which have been so successfully employed in pulmonary tuberculosis are known to have a marked beneficial effect on endobronchial lesions. This would account for the successful and often rapid closure of cavities without collapse measures. It is generally agreed that the bronchus or bronchi draining a cavity are the site of endobronchial lesions. Therefore, it is always essential for the pthisiologist to be aware of an existing endobronchial lesion when he sees a cavity on the roentgenogram. No one will dispute the statement that the fate of a cavity will depend on the fate of the endobronchial lesion.

At this point it is necessary to distinguish between the tension cavity and the cavity which arises wholly by excavation of a sloughing caseous pneumonic lesion. The cavity arising from such excavation represents an absolute loss of parenchymal tissue—an absolute defect. The wall of such a cavity is made up of necrotic caseous material and, on the roentgenogram, the cavity is usually irregular in outline and lies in an area of dense opacity corresponding to the caseous pneumonic lesion. In contrast, on the roentgenogram, the tension cavity is spherical, usually has a relatively thin wall, occasionally a thick wall, and lies in an area of relatively normal parenchyma. If any lesions are seen in the vicinity of a tension cavity, they do not have the appearance roentgenographically of being caseous in character.

If roentgenograms are available before the appearance of the cavity which arises by excavation, the presence of the pre-existing dense opacity charac-

teristic of a caseous pneumonic lesion will be seen. On the other hand, available roentgenograms preceding the appearance of a tension cavity will show a parenchymal lesion much too small in extent to have given rise to the tension cavity now appearing at its site.

The incidence of cavities of significant size which arise wholly by excavation of sloughed tissue is small. Patients with such lesions usually have a poor prognosis because of the extensive caseation and the ease of bronchogenic dissemination. On rare occasions the lesion stops spreading, the caseous tissue sloughs down to healthy granulations, and the remaining defect becomes lined with a healthy layer of smooth fibrous tissue—the so-called "open-cavity healing." Such an absolute defect cannot close or become obliterated as can the tension cavity. It could, conceivably, be obliterated by some measure employing compression aimed to approximate the cavity walls—a true example of collapse therapy. In contrast, the incidence of the spherical type of thin-walled cavity lying in relatively normal lung tissue is much greater, and this is the type of cavity that is seen in the vast majority of roentgenograms in clinical tuberculosis.

Pathogenesis of the Tension Cavity

The pathogenesis of the tension cavity has to do with the establishment of a small tuberculous infiltration in a bronchus and in the surrounding parenchyma supplied by the bronchus. The parenchymal lesion softens and the necrotic material is expectorated, leaving a small hole or defect. The endobronchial lesion in the draining bronchus encroaches on the lumen to such an extent that a check-valve mechanism is set up so that inspired air is trapped during expiration because of shortening and narrowing of the bronchus during the expiratory phase. Thus a tension cavity is formed by the trapped air.

Once formed, the tension cavity enlarges, slowly or rapidly, depending on the severity of cough, rate and depth of respiration, and on the degree of bronchial obstruction. The cavity will cease to enlarge when the pressure built up within is balanced by the elastic recoil of the containing wall which is largely made up of the compressed normal lung tissue (Plate I).

Bearing these mechanics in mind, it is clear that such cavities may arise in relatively short periods of time, measured in weeks or even days, and that the cavities may disappear in short periods of time if the bronchial obstruction is completely relieved. If the bronchial obstruction is relieved, the trapped air will escape, the cavity become smaller, the supra-atmospheric pressure within the cavity fall, and the compressed normal parenchyma which made up the cavity wall become aerated, thus obliterating the cavity. Monaldi explained the obliteration of large cavities following catheter drainage by a similar mechanism, namely, reduction of pressure within the cavity by suction, and obliteration by re-expansion of compressed parenchymal tissue which made up the wall of the tension cavity. Since large tension cavities may arise at the site of small defects, it is not surprising that small scars are seen on roentgenograms after obliteration and healing of such cavities.

Tension cavity healing: Tension cavities may heal in two ways, i.e., by complete obliteration and minimal scarring and by inspissation.

Healing by obliteration: If the endobronchial obstruction is completely relieved with escape of the trapped air and cavity contents, the cavity will be obliterated, the opposing lining walls of the cavity will approximate, and the line of approximation will be replaced by a linear scar. This is ideal healing and it occurs often.

Healing by inspissation: Inspissation occurs if the draining bronchus becomes completely obstructed by scar tissue, heaped up infiltrations, intraluminal debris or edema before the cavity contents have had a chance to escape. Under such circumstances, cavity gas is absorbed, the cavity becomes smaller, but the retained secretions and debris are trapped and undergo inspissation. The inspissated area is smaller than the original cavity and on the roentgenogram there is an opacity which shows little change over a long period of time in a patient who has sputum negative for tubercle bacilli and whose lesion is clinically—although not anatomically—inactive. Loesch (9) and Auerbach and Green (10) have published microscopic sections showing cavity healing by the ideal method, which is by obliteration and minimal scarring, and by inspissation.

The following case reports are illustrative of various aspects of this problem of tension cavities.

Case Reports

Case 1. H.L.H. (Reg. No. 60306) was a white male, 43 years old, who was admitted to the Veterans Administration Hospital at Oteen with a short history of acute symptoms that began January 25, 1951. In 1919, he had "double pneumonia" and influenza. At the time of induction into the Army in 1943, a chest roentgenogram showed a fibrotic lesion in the right apex, apparently inactive. A routine chest roentgenogram in July, 1950 showed no change since 1943. About January 25, 1951, the patient had a "deep chest cold" which followed a duck hunt in damp cold weather. This cold was accompanied by soreness across the right upper chest and fairly severe cough productive of thick dark sputum. On February 4, 1951 he began to expectorate blood-streaked sputum. He continued to have "streaked" sputum for four days and the chest soreness continued for about ten days. The patient sought medical aid on February 5, 1951, because of the "streaking" of the sputum. A roentgenogram on that day showed the lesion in the right rib circle and first interspace, and comparison with the July, 1950, roentgenogram revealed an increase in the extent of the lesion. Hospitalization was advised.

The patient entered the Oteen Veterans Administration Hospital on February 25, 1951. A roentgenogram on February 26, 1951, revealed a 2 cm. cavity behind the right first rib (Plate II). The sputum was positive for acid-fast bacilli on microscopy. The patient was given 0.5 gm. of streptomycin and 12 gm. of PAS daily along with a low carbohydrate high protein diet. Good progress was made clinically and serial monthly roentgenograms showed steady clearing of the lesion with gradual reduction in the size of the cavity. The roentgenogram of May 10, 1951, showed no evidence of cavity. Planigrams showed ideal healing with minimal scarring. The patient completed the course of treatment which included physical rehabilitation and has remained well since.

Comment: The patient had an old scar in the right apex, probably tuberculous in origin. There was reactivation of the lesion at the time of the severe chest cold. The roentgenogram on February 5, 1951 showed no evidence of cavity but

did show evidence of activity. The patient had several days of sputum "streaking" and chest soreness. A roentgenogram on February 26, 1951, showed a 2 cm. cavity.

It is very likely that a tuberculous endobronchitis was present soon after the onset of the patient's illness. The endobronchial lesion acted as a check-valve with subsequent development of a tension cavity which was seen on the roentgenogram of February 26, 1951. This cavity thus appeared during the twenty-one-day period between the roentgenograms of February 5, 1951, and February 26, 1951. Since there was no evidence of caseous pneumonic disease on the roentgenogram of February 5, 1951, the appearance of the cavity cannot be explained on the basis of sloughing and excavation. Its rapid development can be explained by implicating a check-valve mechanism with tension cavity formation. The severe cough could readily hasten the development of tension cavity. The "streaking" of the sputum might be attributed in part to the tearing of alveolar septa by the supra-atmospheric pressures built up by the trapped air. The chest soreness might also be due to the stretching and tearing of elastic and connective tissue during the tension cavity formation.

Case 2. L.A.R. (Reg. No. 54086), was a white male, 31 years old, who was admitted to the Veterans Administration Hospital at Oteen on October 11, 1949, because a cavity was found on a routine chest roentgenogram on September 11, 1949. (Plate III). While on duty with the Army in 1941, the patient had incision and drainage of a right scrotal abscess which proved to be tuberculous. This lesion healed without complication. At that time a chest roentgenogram revealed a small opacity in the left apex which was regarded as inactive. He returned to duty and was discharged in September, 1945. Yearly routine chest roentgenograms thereafter showed no change until September 11, 1949, when a cavity was seen at the site of the hitherto inactive lesion. The veteran had the usual symptoms of hay fever at the time, namely, sneeze, cough, and wheeze. The hay fever had been recurring annually for several years. He was admitted to Oteen on October 11, 1949, and a 2 cm. cavity was seen at the level of the left second anterior rib. The sputum was positive for acid-fast bacilli on culture. The patient was put on a low carbohydrate high protein diet and bed rest. No chemotherapy was given because at that time only selected patients were given the antituberculous drugs. The patient's disease responded favorably, and a roentgenogram on December 11, 1949, showed cavity closure with replacement by a small solid nodule. He completed the full course of treatment, including physical rehabilitation, and has remained well ever since.

Comment: This patient had an inactive fibrotic nodular lesion in the left apex for several years, undoubtedly tuberculous in origin. A routine roentgenogram in September, 1949, revealed a 2 cm. spherical cavity at the site of the nodular lesion. He had no unusual symptoms at that time other than the cough, wheeze, and sneeze due to seasonal hay fever. It is very likely that the mucosal edema due to hay fever, combined with a reactivation of the tuberculous focus, produced an endobronchial obstruction which set up a check-valve mechanism. The combination of endobronchitis, mucosal edema, cough, and sneezing readily caused air trapping with tension cavity formation.

Case 3. Acute tension cavity following pneumoperitoneum and phreniclasia. D.C.S. (Reg. No. 49895) was a white male, 37 years old, who was admitted to the Veterans Administration Hospital at Oteen on October 24, 1948, with active pulmonary tuberculosis, first diag-

nosed in August, 1948. He had slight symptoms. Pneumoperitoneum was instituted on November 4, 1948. The patient improved gradually on this regimen until April 6, 1949, when he began to show heavy "streaking" of the sputum. A roentgenogram revealed a spread of the disease. The pneumoperitoneum was stopped temporarily. Streptomycin, 1 gm. every third day, was begun on April 18, 1949. A roentgenogram on July 11, 1949, showed a 1.5 cm. cavity in the left second interspace anteriorly, and no evidence of pneumoperitoneum was seen (Plate IV). Because of the spread of the disease and the presence of the cavity, a left phreniclasia was done on August 4, 1949, and pneumoperitoneum was reinstituted on August 5, 1949.

On August 12, 1949, the patient had sudden chills and fever. On August 13, 1949, heavy "streaking" of the sputum occurred and kept up for four days. A roentgenogram on August 16, 1949, showed a marked increase in the size of the cavity in the left lung, atelectasis of the lower lobe, and pneumoperitoneum with considerable elevation of the left leaf of the diaphragm. Pneumoperitoneum was discontinued at once. A roentgenogram on August 26, 1949, showed a slight reduction in the size of the cavity, with some lowering of the left leaf of the diaphragm. A roentgenogram on September 16, 1949, showed marked reduction in the size of the cavity, and pneumoperitoneum was no longer evident. The atelectasis had disappeared. The left leaf of the diaphragm was lower than it was on the August 16, 1949, roentgenogram, but was still elevated because of the phreniclasia.

Comment: This patient had sudden onset of chills, fever, and heavy streaking eight days after a successful phreniclasia and institution of pneumoperitoneum. A roentgenogram revealed marked increase in the size of the cavity with fluid level, along with atelectasis of the lower lobe. Following discontinuation of the pneumoperitoneum there was prompt reduction in the size of the cavity and clearing of the atelectasis. It is very likely that the elevation of the left leaf of the diaphragm produced by the pneumoperitoneum and phreniclasia exerted sufficient compression on the bronchus draining the cavity. The upward pressure and subsequent compression, added to an endobronchial lesion, set up a check-valve mechanism that caused an acute tension cavity. The discontinuation of pneumoperitoneum resulted in a lowering of the left leaf with rapid reduction in the size of the cavity. The heavy "streaking" of the sputum that occurred after the phreniclasia and institution of pneumoperitoneum could be attributed to severe stretching and tearing of parenchyma and elastic tissue which accompanied the sudden ballooning of the cavity. The chills and fever could be attributed to the atelectasis which likewise resulted from bronchial compression. Although the phreniclasia caused complete paralysis and elevation of the leaf of the diaphragm, the discontinuation of the pneumoperitoneum lowered the leaf sufficiently to relieve the bronchial compression.

Case 4. Tension cavity developing during pneumoperitoneum. E.D.R. (Reg. No. 55546) was a white male, 25 years old, who had tuberculosis since February, 1945. He was treated with bed rest and bilateral pneumothorax at the U. S. Naval Hospital, Sampson, New York, from June, 1945, to June, 1946. He was discharged as having inactive tuberculosis in August, 1948. He was admitted to the Veterans Administration Hospital at Oteen on March 4, 1949, because of a reactivation of his disease. Pneumoperitoneum was instituted on March 10, 1949, because of a cavity in the right lung. The patient left the hospital against medical advice on August 4, 1949. He continued taking pneumoperitoneum refills as an outpatient at Oteen. A roentgenogram on July 27, 1949, showed the presence of pneumoperitoneum with no evidence of cavity (Plate V). A routine roentgenogram on February 9, 1950, revealed a large spherical cavity in the right midlung surrounded by relatively normal lung parenchyma. Pneumoperitoneum was present, with considerable elevation of the dia-

phragm. He was admitted to the hospital. Pneumoperitoneum was promptly discontinued. Streptomycin, 1 gm. and PAS, 12 gm., were given daily from February 24, 1950. Bronchoscopy on March 7, 1950, revealed evidence of endobronchial disease in the right lung. A roentgenogram on May 23, 1950, showed no evidence of cavity, and pneumoperitoneum was not present.

Comment: The development of the tension cavity during the course of pneumoperitoneum may be attributed to a check-valve mechanism in the draining bronchus. The upward pressure of the right leaf of the diaphragm was sufficient to compress the bronchus. The combination of endobronchial disease and mechanical compression resulted in the development of a large tension cavity. The discontinuation of pneumoperitoneum and the administration of streptomycin and PAS were sufficient to relieve the endobronchial obstruction. As a result, the tension cavity disappeared after a short time.

Case 5. Tension cavity in contralateral lung following pneumonectomy. W.E.N. (Reg. No. 59372) was a white male, 32 years old, who was admitted to the Veterans Administration Hospital at Oteen on December 6, 1950. In spite of vigorous chemotherapy and pneumoperitoneum, the course of the disease was steadily downhill. The disease in the left lung cleared readily, but the right lung was destroyed. A right pneumonectomy was performed on July 24, 1952, with completion of thoracoplasty, and he made an uneventful recovery. Bronchoscopy on June 23, 1952, showed no evidence of endobronchial disease in the visible left bronchial tree; and bronchoscopy on October 27, 1952, was likewise negative. On December 2, 1952 (Plate VI), a roentgenogram showed a small productive lesion with cavity in the first anterior interspace of the left lung which, prior to pneumonectomy, was considered free of active disease. Serial roentgenograms showed a progressive increase in the size of the cavity which had characteristics of a tension cavity. The cavity is present at the time of writing this article, January, 1957, and it is 5 cm. in diameter. It has not responded to varied combinations of chemotherapy and it fluctuates in size over a narrow range.

Comment: The origin of this tension cavity in the apparently uninvolved left lung after right pneumonectomy can be readily explained by a check-valve mechanism resulting from the mediastinal shift with distortion of the bronchial tree radiating from the left hilum. This shift is apparent on the postoperative roentgenograms. There was no caseous lesion in the left lung which could have excavated to produce the cavity. Serial roentgenograms show steady inflation and ballooning of the cavity over a period of several months. No doubt there was an endobronchial lesion present at the time of surgery, but it was beyond the reach of the bronchoscope. The bronchial distortion accompanying the mediastinal shift probably caused a kink in the draining bronchus, which, added to the endobronchial obstruction caused by the tuberculous lesion produced the check-valve mechanism responsible for the cavity. The persistence of the cavity for over four years, and the failure to respond to chemotherapy indicate that it is due chiefly to irreversible mechanical factors rather than to endobronchial tuberculosis.

Discussion

Most cavities seen on the roentgenogram in clinical tuberculosis are undoubtedly of the tension variety which arise originally as a small defect in the parenchyma resulting from the excavation of a necrotic focus. Subsequent en-

largement of the cavity, in the absence of a progressive caseating lesion to account for further enlargement, is due most likely to a check-valve mechanism producing the tension cavity. The check-valve may be due entirely to an endobronchial lesion causing obstruction or it may be due to a mechanical factor. Usually the obstruction appears to be the result of a combination of the two factors. Collapse measures such as pneumothorax, pneumoperitoneum, phreniclasia, and thoracoplasty, may cause bronchial obstruction by external compression of the bronchi.

It is obvious that moderate compression or distortion will readily produce obstruction in a terminal bronchiole which is the site of an endobronchial lesion. Also, distortion of the bronchial tree may readily follow significant mediastinal shift after pneumonectomy, lobectomy, and segmental resection. Acute or chronic atelectasis, by causing compensatory shift of mediastinal structures and/or shift of the remaining functioning lobes, can also lead to bronchial distortion. Moreover, it would appear that the bronchi radiating upwards to the upper lobes would be more vulnerable to such distortion because of their angulation. Therefore, in planning therapy for any cavity, it is essential to bear in mind the state of the draining bronchus. Therapy should, whenever feasible, bring about relief of the obstruction.

There are cases with roentgenographic characteristics of tension cavity located in the extreme apex which fail to respond to chemotherapy and bed rest even though drug-susceptible tubercle bacilli are present. It is safe to assume that, if an endobronchial lesion were present, it would have responded to adequate chemotherapy with relief of the endobronchial obstruction and removal of the check-valve mechanism. Goldman and associates (11) have suggested an excellent explanation of why such apical cavities fail to collapse like tension cavities located in other parts of the lung. They believe that the apical pleura forms a portion of the cavity wall and is held by adhesions over a broad base to the parietal pleura. Hence, the cavity is unable to close even though the bronchial obstruction has been removed. The needling of such cavities will show atmospheric pressures because the bronchus is patent.

Examination of the lung at autopsy does not give a true picture of the tension cavity as it existed during life when it was subjected to forces that no longer operate. Then, the inspired air was being trapped distal to the check-valve in sufficient quantity to keep the cavity inflated at supra-atmospheric pressure. Also, the wall of the tension cavity is made up largely of compressed normal parenchyma, and equilibrium is maintained between the intracavitary pressure and the elastic recoil of the tense cavity wall which has a constant tendency to expel the trapped air through the draining bronchus. These opposing forces are responsible for the size and configuration of the tension cavity as it is seen on the roentgenogram during life. At autopsy the true picture is lost; the tension cavity will be much reduced in size due to (*1*) cessation of breathing and no more air trapping; (*2*) absorption of air that had been trapped during life; and (*3*) possible escape of trapped air through the draining bronchus. Thus a cavity that was present during life may not be found or is found much reduced in size, especially if the cavity is of recent origin. A chronic ten-

sion cavity is more likely to hold its shape at autopsy because of a thicker fibrotic wall.

Conceivably, the mechanisms which can produce tension cavities in pulmonary tuberculosis can also produce them in any type of lesion that can cause focal parenchymal infiltration with accompanying bronchial lesion, such as abscess, coccidiodomycosis, and blastomycosis. Bronchial obstruction occurring in asthma, chronic bronchitis, or any disease or disorder accompanied by bronchial narrowing, may also lead to air trapping and thus produce emphysema, cysts, and blebs.

Summary

Tension cavities are often encountered in pulmonary tuberculosis. The cavities are caused by a check-valve mechanism resulting chiefly from two factors: an organic bronchial obstruction or a mechanical bronchial obstruction. Most often there is a combination of the two factors. In planning therapy for patients with tension cavity it is important to keep in mind the fundamental relationship between the obstructive mechanism in the draining bronchus and the development, persistence, and obliteration of the tension cavity. The plan of therapy should be directed toward the relief of the bronchial obstruction.

The concept of the tension cavity is valuable because it helps explain several clinical phenomena, such as, the development of a cavity in a short period of time; the development of cavity in an area of lung that had not been the site of a caseous lesion capable of excavating and giving rise to cavity; the fluctuation in size, with or without fluid level, at irregular intervals; variations in amount and character of the sputum; transient or permanent blocking of the cavity with replacement of cavity gas by secretions; the origin of sputum "streaking" and localized chest pain or soreness during the development of the tension cavity; and the rapid closure of cavity and healing with a minimal scar.

Sumario

Patogenia y Tratamiento de las Cavernas Valvulares del Pulmón

En la tuberculosis clínica se observan a menudo cavernas infladas, producidas por un mecanismo de válvula de retención, que proviene principalmente de dos factores: una oclusión bronquial orgánica o una oclusión bronquial mecánica. Lo más frecuente es una combinación de los dos factores. Al proyectar la terapéutica para los enfermos con cavernas valvulares, es importante tener presente la relación fundamental que existe entre el mecanismo oclusivo del bronquio de irenaje y la formación, persistencia y obliteración de la caverna inflada. El plan de terapéutica debe encaminarse al alivio de la obstrucción bronquial.

El concepto de la caverna valvular es valioso porque ayuda a explicar varios fenómenos clínicos, tales como: la formación de una caverna en un breve período de tiempo; la formación de caverna en una zona pulmonar que no había sido asiento de una lesión caseosa capaz de excavarse y de producir cavitación; la fluctuación del tamaño, con o sin nivel de líquido, a plazos irregulares; variaciones de la cantidad y la naturaleza del esputo; el bloqueo transitorio o permanente de la caverna, reemplazando las secreciones el gas de aquélla; el origen del dolor o de la hiperestesia viariables y localizados en el tórax durante la formación de la caverna valvular; y el rápido cierre de la caverna y su curación con una cicatriz mínima.

Resume

Pathogénie et traitement des cavernes pulmonaires sous tension

Les excavations sous tension sont souvent rencontrées dans les cas cliniques de la tuberculose. Elles sont causées par un mécanisme de clapet résultant de deux facteurs: l'obstruction bronchique organique ou l'obstruction bronchique mécanique. La plupart du temps il y a une combinaison des deux facteurs. Dans l'établissement d'un plan thérapeutique destiné aux patients porteurs de cavernes sous tension, il est important de se souvenir de la relation fondamentale existant entre le mécanisme d'obstruction au niveau de la bronche de drainage et le développement, la persistance et l'oblitération de l'excavation sous tension. Le programme thérapeutique doit être dirigé vers la libération de l'obstruction bronchique.

La conception de la caverne sous tension a de la valeur car elle aide à expliquer certains phénomènes cliniques, tels que: le développement d'une caverne à bref délai; le développement d'une caverne dans une zone n'ayant pas été le siège d'une lésion caséeuse apte à s'excaver et à produire une caverne; la fluctuation des dimensions, avec ou sans niveau liquidien, à intervalles irréguliers; les variations de la quantité et du caractère de l'expectoration; l'oblitération transitoire ou permanente de la caverne avec remplacement des gaz cavitaires par des exudats; les douleurs thoraciques fulgurantes localisées ou les algies durante le développement de la caverne sous tension; l'oblitération rapide de la caverne et sa cicatrisation avec cicatrice minime.

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Plates I–VII will follow

PLATES I–VII

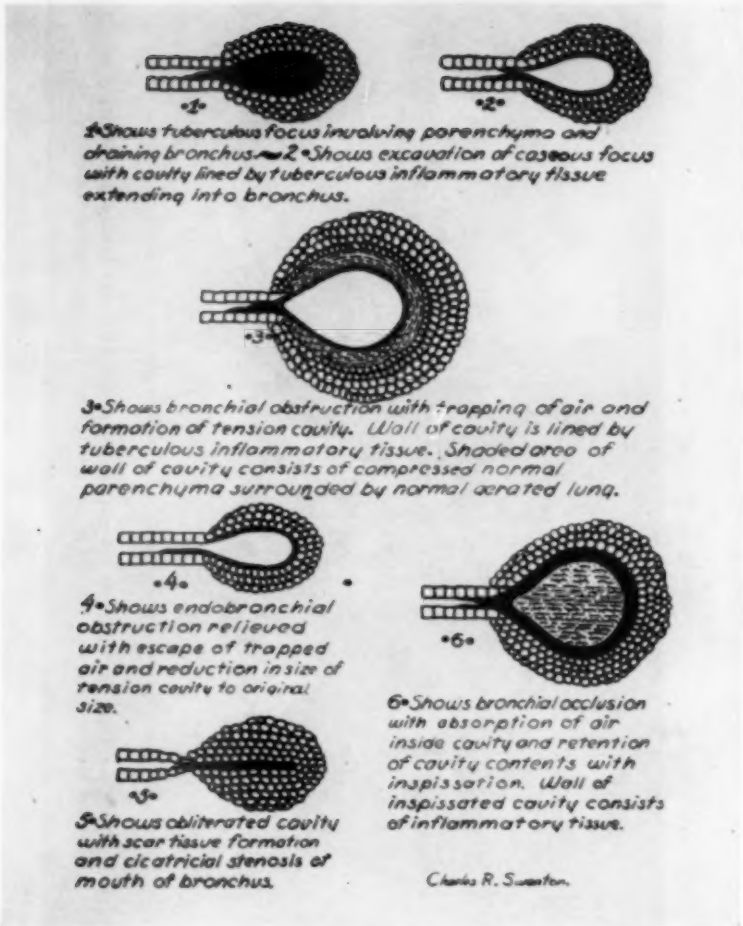
1. Shows tuberculous focus involving parenchyma and draining bronchus. 2. Shows excavation of caseous focus with cavity lined by tuberculous inflammatory tissue extending into bronchus.
3. Shows bronchial obstruction with trapping of air and formation of tension cavity. Wall of cavity is lined by tuberculous inflammatory tissue. Shaded area of wall of cavity consists of compressed normal parenchyma surrounded by normal aerated lung.
4. Shows endobronchial obstruction relieved with escape of trapped air and reduction in size of tension cavity to original size.
5. Shows obliterated cavity with scar tissue formation and cicatricial stenosis of mouth of bronchus.
6. Shows bronchial occlusion with absorption of air inside cavity and retention of cavity contents with inspissation. Wall of inspissated cavity consists of inflammatory tissue.
Charles R. Swanton.

PLATE I

Schematic drawings to describe the possible steps in the formation of a tension cavity from a parenchymal defect or "hole" resulting from the sloughing of a caseous necrotic focus (figures 1–4); and drawings to describe the two methods of cavity closure and healing (figures 5, 6).

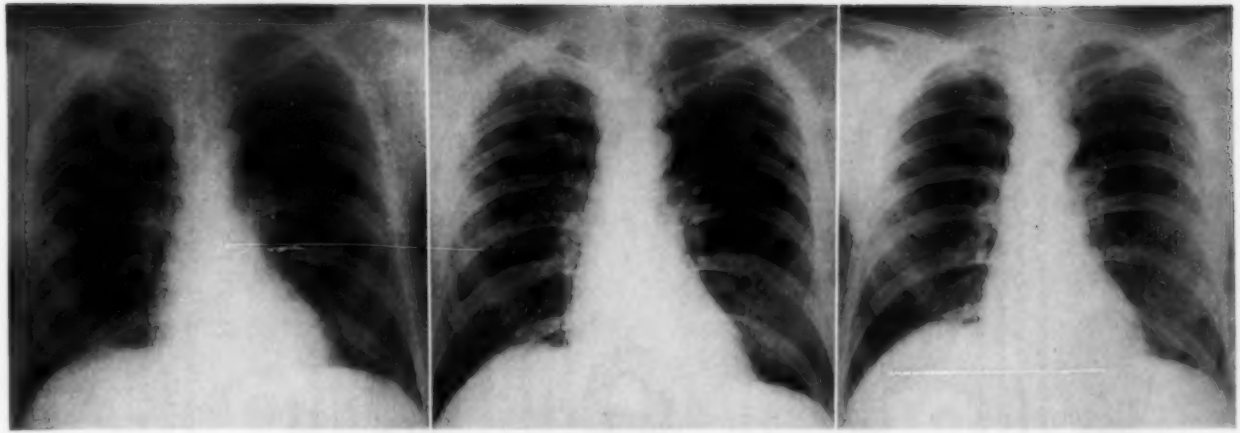

PLATE II

Case 1 (H.L.H.)

FIG. 1 (Left). Roentgenogram taken on February 5, 1951, shows an infiltration in the right rib circle and first anterior interspace without cavity.

FIG. 2 (Center). Roentgenogram taken twenty-one days later on February 26, 1951, shows a cavity behind the right first rib anterior.

FIG. 3 (Right). Roentgenogram taken on May 24, 1951, shows cavity to be closed. There has been clearing of the infiltrate.

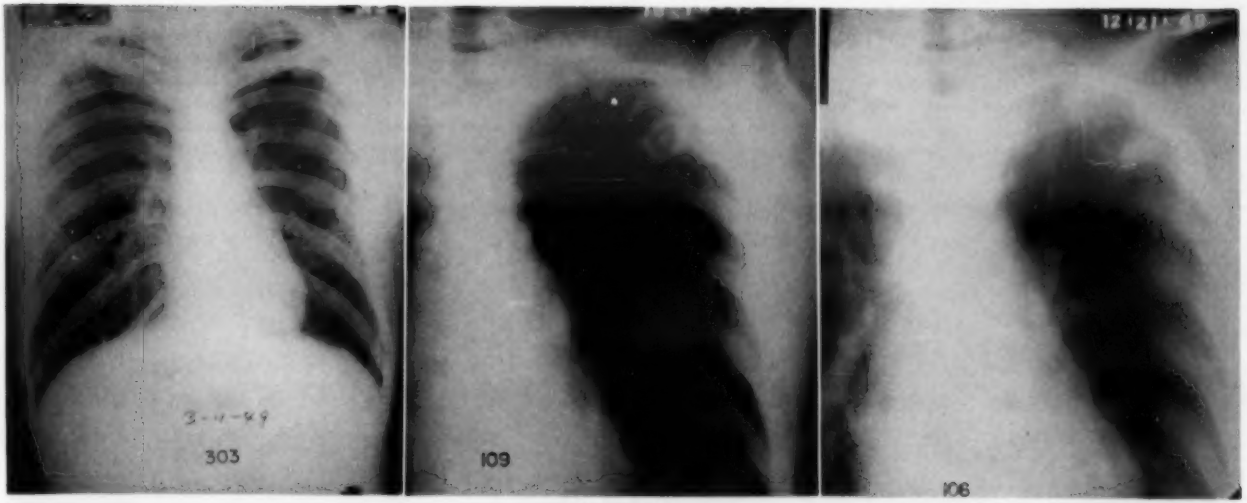

PLATE III

Case 2 (L.A.R.)

FIG. 1 (Left). Roentgenogram taken on March 11, 1949, shows linear productive lesion in the left first interspace anteriorly which had been present since 1941.

FIG. 2 (Center). Roentgenogram (planigram), taken on October 15, 1949, shows the cavity in the left first interspace anteriorly.

FIG. 3 (Right). Roentgenogram (planigram) taken on December 21, 1949, shows no evidence of cavity in the left lung. A solid nodule is seen at the site of the former cavity.

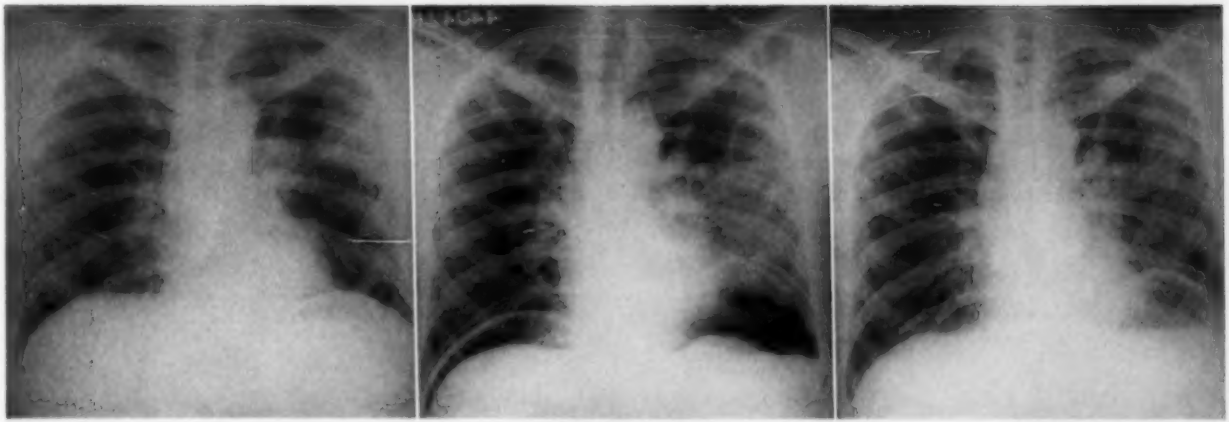

PLATE IV

Case 3 (D.C.S.)

FIG. 1 (Left). Roentgenogram taken on July 11, 1949, shows a 1.5 cm. cavity in the left second interspace anteriorly, prior to phreniclasia and induction of pneumoperitoneum on August 4, 1949, and August 5, 1949, respectively.

FIG. 2 (Center). Roentgenogram taken on August 16, 1949, twelve days after left phreniclasia, shows elevation of the left leaf of the diaphragm, pneumoperitoneum, and presence of acute tension cavity. The lower half of the left lung is atelectatic.

FIG. 3 (Right). Roentgenogram taken on September 16, 1949, shows marked reduction in the size of the cavity and clearing of the atelectasis. Pneumoperitoneum is not present. Moderate elevation of the left leaf of the diaphragm, due to phrenic paralysis, persists.

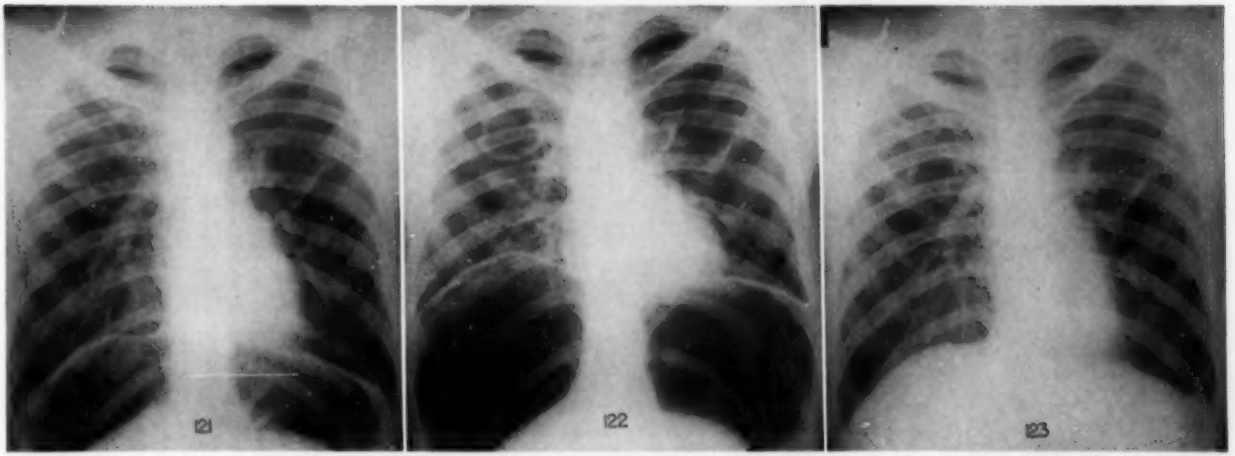

PLATE V

Case 4 (E.D.R.)

FIG. 1 (Left). Roentgenogram taken on July 27, 1949, shows bilateral linear and nodular productive lesions with no evidence of cavity. Pneumoperitoneum is present. At this time the patient was receiving refills of pneumoperitoneum in the outpatient department.

FIG. 2 (Center). Roentgenogram taken on February 9, 1950, during a routine examination. A large tension cavity is seen in the right midlung. Pneumoperitoneum is greater than on the previous film and the right leaf of the diaphragm is higher.

FIG. 3 (Left). Roentgenogram taken on May 23, 1950, shows that pneumoperitoneum has been absorbed and the leaves of the diaphragm are in normal position. The large tension cavity previously seen in the right midlung is not present.

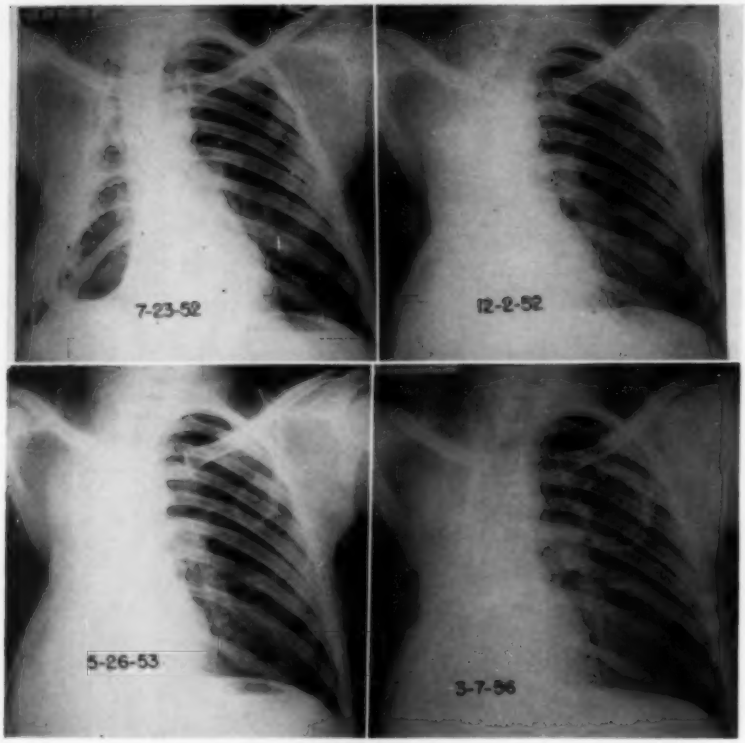

PLATE VI

Case 8 (W.E.N.)

FIG. 1 (Upper left). Roentgenogram taken on July 23, 1952, before pneumonectomy and completion of thoracoplasty. The left lung shows slight linear productive changes with no evidence of cavity.

FIG. 2 (Upper right). Roentgenogram taken on December 2, 1952, shows post-pneumonectomy status. There has been considerable shift to the right of the heart and mediastinal structures. A cavity is now seen in the region of the left second rib anterior.

FIG. 3 (Lower left). Roentgenogram taken on May 26, 1953, shows further enlargement of the cavity in the left lung.

FIG. 4 (Lower right). Roentgenogram taken on March 7, 1956, shows the cavity in the left lung to be larger, with the characteristics of a tension cavity.

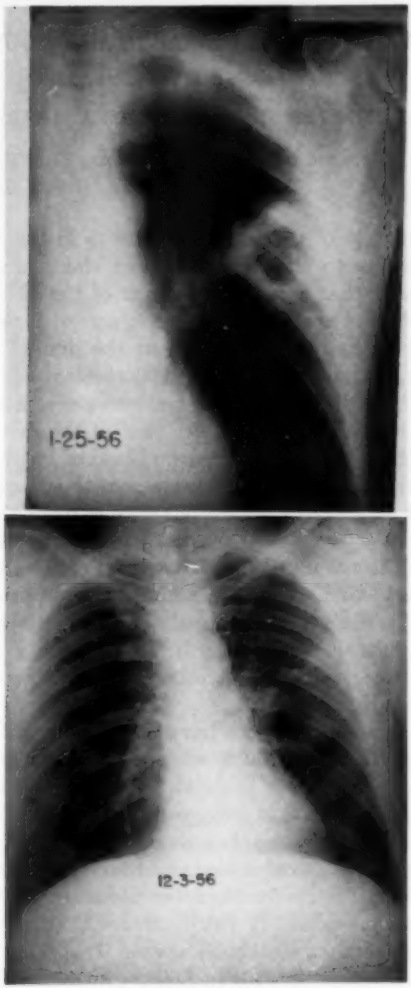

PLATE VII

FIG. 1. Planigram of a tension cavity to illustrate the almost uniform thickness of the wall and the uniform ground-glass opacity cast by the wall of a cavity on the roentgenogram. These roentgenographic features are most likely due to the belief that the wall is made up of compressed airless normal parenchyma. This cavity wall is thicker than those usually seen.

FIG. 2. Roentgenogram of lungs of the same patient shown in figure 1. It was taken on December 3, 1956. The cavity is almost closed. Healing is occurring by the ideal method of obliteration resulting from clearing of bronchial obstruction and minimal scarring.

PRIMARY TUBERCULOSIS IN INFANTS AND YOUNG CHILDREN[1]

Survey of a Slum Area in Puerto Rico

JOSE E. SIFONTES[2] AND ANASTASIOS A. ANASTASIADES

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INTRODUCTION

A previous survey (1) of the pediatric patients in the Alejandro Ruiz Soler Sanatorium, where Puerto Rican infants and children with tuberculosis are hospitalized, revealed that a large percentage of the children (24.6 per cent) were zero to two years of age. Most of these infants with tuberculosis came from the slum areas of San Juan, particularly from the slum of La Perla, which had contributed the largest number of tuberculous children less than twelve years of age. The patients from this area represented 80.8 per cent of hospitalized tuberculous children from San Juan, 31.8 per cent of the metropolitan area total, and 12.9 per cent of the total from the entire island of Puerto Rico. Furthermore, 65 per cent of patients from La Perla were zero to two years of age, whereas, only 24 per cent of all pediatric admissions were in this age group.

In a study (2) of tuberculous meningitis in children admitted to the San Juan City Hospital, it was noted that 53 per cent of the patients were less than two years of age and 67 per cent were less than four years of age. The occurrence of meningitis in these children so early in life clearly indicated close contact with tuberculous adults shortly after birth. The possibility of bovine tuberculosis may be discarded since this form of tuberculosis has been almost completely eradicated in Puerto Rico.

In recent years, these observations have been confirmed by the study of more cases, including all children with tuberculous meningitis from San Juan and surrounding areas. In figure 1 it is shown that the predominant age group for tuberculous meningitis is less than four years and that a high incidence of meningitis is observed even in those less than six months old.

In a preliminary report (3) of a controlled investigation in San Juan on the effectiveness of isoniazid in preventing tuberculous meningitis and other complications of primary tuberculosis, it was concluded that more patients should be observed in order to obtain a significant answer. This study was stimulated by a similar investigation (4) by Dr. Edith Lincoln in the Children's Chest Service of Bellevue Hospital. Now a large-scale cooperative investigation coordinated by the U. S. Public Health Service is being carried out in a number of pediatric centers, including the Alejandro Ruiz Soler Sanatorium.

Based on previous observations, it seemed likely that a systematic search for

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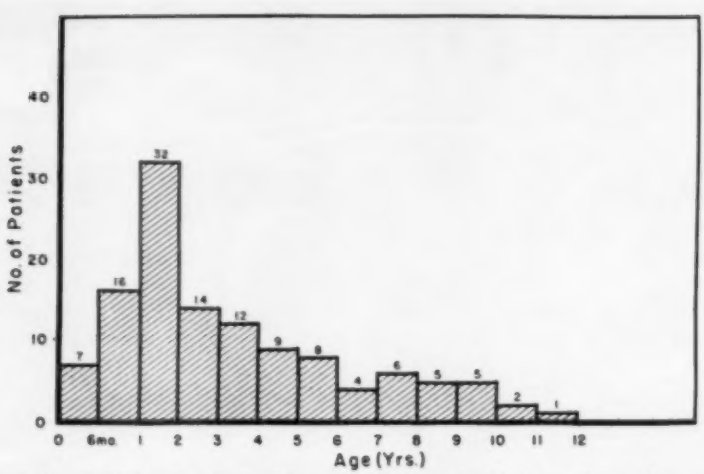

FIG. 1. Age of children hospitalized for tuberculous meningitis in San Juan and surrounding areas. The predominant age group is less than four years and, even in those less than one year, the incidence is high.

young patients with recently acquired tuberculosis in the slum of La Perla would yield a large number of suitable patients for the USPHS study. The present report presents the results of a house-to-house tuberculin survey in La Perla.

MATERIALS AND METHODS

Description of the Slum of La Perla

The slum of La Perla, about 1,000 feet long and 400 feet wide, consists of a cluster of closely packed huts of wood and cardboard, precariously perched on a cliff on the north shore of the island city of San Juan (figures 2, 3). Its streets are narrow alleys 2 to 3 feet wide (figure 4), opening on three main avenues 15 feet wide. This small area is densely populated with 1,300 families averaging 5 children per family.

A large number of tuberculous adults live in this area; many are under treatment with isoniazid and streptomycin at the tuberculosis centers, others are delinquents from the government sanatoriums which they have left against medical advice, and still others are too apathetic to go the public health units or tuberculosis centers for free diagnosis and treatment.

As soon as the children learn to walk, they roam from one house to another and promptly become infected with tuberculosis, dysentery, and parasites. Their diet consists mainly of rice, sometimes supplemented by codfish or beans. Most of the protein that they obtain is supplied by the free government milk stations and breakfast centers provided by the insular and municipal governments.

It is a major expense to go to the health unit or center and the children are normally brought for medical care only when they appear dangerously ill or when the importance of the visit to the clinic is made urgently clear to the parents. Fortunately, the slum of La Perla is being gradually eliminated and numerous families already have been moved to modern housing projects (figure 5).

Age: The survey was limited to children less than four years of age because previous

FIG. 2. Aerial view of the slum of La Perla illustrating the large number and close proximity of the houses.

experience had indicated that most older children from such slums had calcified pulmonary lesions.

Method of making the survey: Each house in La Perla was visited twice, first for application of the tuberculin and again to read the test. Because of the difficulties involved in house-to-house tuberculin testing, it was decided to use the Vollmer patch test initially, and to retest the positive reactors with PPD in the outpatient department of the Sanatorium. The parents were instructed to remove the patch after forty-eight hours, and the reaction was observed forty-eight hours later.

The dose of PPD was 0.0001 mg. (intermediate, Sharp and Dohme). The reaction was observed forty-eight hours later and was recorded in terms of millimeters of induration; an area of induration greater than 5 ml. in diameter was considered positive.

At the time when the intradermal tuberculin test was read, chest roentgenograms, postero-anterior and lateral, were obtained for all positive reactors. The roentgenographic findings were categorized, as follows: (*1*) active primary tuberculosis; enlarged hilar nodes or density in the lung parenchyma, or both, without calcifications; (*2*) healing primary tuberculosis, evidence of active primary tuberculosis containing calcifications; (*3*) healed primary tuberculosis, hilar or parenchymal calcifications without visible active lesions; and (*4*) negative: (*a*) questionable hilar enlargement, (*b*) doubtful calcification, (*c*) no lesion observed.

RESULTS

Five hundred and twenty-five children were tested with tuberculin by the Vollmer patch method between February 14 and March 2, 1955. Four hundred and seventy-five were located for reading the reaction. One hundred and seventy-seven children showed positive patch reactions and, of these, one hundred and seventy (35.8 per cent of the total number located) were confirmed by intradermal tuberculin reactions of more than 5 mm. of induration to 0.0001 mg. of

FIG. 3. Most of the houses in the slum have been hastily constructed of any available material, such as wood, tin, cardboard, and occasionally concrete.

FIG. 4. Most of the streets are narrow alleys, two to three feet wide, winding between houses like a labyrinth and finally opening up on three, fifteen-foot avenues.

FIG. 5. A large part of La Perla slum already has been eliminated and inhabitants moved to these modern apartment buildings. This program is continuing, and it is expected that the entire slum will disappear.

TABLE 1

RESULT OF TUBERCULIN SURVEY IN LA PERLA, PUERTO RICO

	Number and Per Cent of Positive Tuberculin Reactors						
	Total	Age in Years					
		Less than 1	2	3	4	5	Unknown
Total tested and read	475	153	116	147	42	9	8
Positive reactors	170	16	30	83	31	8	2
Per cent of positive reactors	35.8	10.4	25.9	56.5	73.8	88.9	

PPD. Analysis of positive reactors in the various age groups is shown in table 1. One of 10 infants less than one year of age, and one of 4 between the ages of one and two years had positive tuberculin reactions. More than 60 per cent of the children in the age group three to five years had positive reactions to tuberculin. This sharp rise in the incidence of positive reactors probably reflects increased exposure of older children outside their own homes.

The size of the reaction was recorded in all but 32 cases. Most of the children, even the very young ones, appeared to be hypersensitive to PPD, showing reactions greater than 10 mm. in diameter (table 2).

Roentgenograms of the positive reactors (table 3) showed that 54.3 per cent of children less than two years of age had active primary lesions. This figure comes close to the findings of Brailey (5), who estimated that roentgenographic

TABLE 2

SIZE OF INDURATION IN MILLIMETERS OF THE CUTANEOUS REACTION TO PPD

	Induration				
	5-9 Mm.	10-14 Mm.	15-19 Mm.	20 Mm. and More	Positive PPD (Size of Induration Not Recorded)
Number of positive reactors	2	47	60	29	32
Per cent of total positive reactors	1.2	27.6	35.3	17.1	18.8

TABLE 3

ROENTGENOGRAPHIC FINDINGS AMONG POSITIVE REACTORS TO TUBERCULIN

Roentgenographic Classification	Age of Child			
	Less than 2 Years		2-5 Years	
	Number	Per Cent	Number	Per Cent
Total	46	100.0	124	100.0
Active lesions	25	54.3	31	25.0
Parenchymal	1		1	
Mediastinal nodes	17		27	
Parenchymal and mediastinal nodes	7		3	
Healing	—		12	9.7
Healed	—		30	24.2
Negative	21	45.7	51	41.1

abnormalities occurred in 61 per cent of children less than two years of age with positive tuberculin reactions (1939). Lincoln (6) reported roentgenographic abnormalities in almost 90 per cent of reactors in the same group (1950). In the present study, the roentgenograms of children two to five years of age revealed that 25 per cent had active primary lesions, 10 per cent had healing primary lesions, and 24 per cent had healed calcified primary tuberculosis. A total of 59 per cent of positive reactors two to five years of age had roentgenographic evidence of tuberculosis.

COMMENT

Detection of primary tuberculosis in its early stages is important because most of the serious complications such as tuberculous meningitis occur within the first year after inital infection (7). In San Juan and the surrounding areas, tuberculous meningitis has been observed most frequently in children less than four years of age. This observation coincides with the high prevalence of tuberculous infection among infants and young children in La Perla.

The number of children tested in this survey is small when compared with that of other surveys, but the results show high prevalence of tuberculosis in a

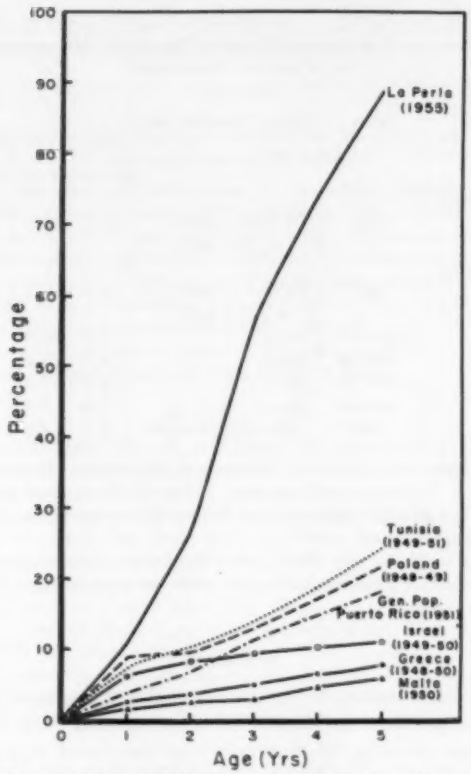

FIG. 6. Tuberculin sensitivity of children less than five years of age from La Perla compared with that of children of the same age in the general population of Puerto Rico and other parts of the world.

very young age group in a selected area, when compared with surveys in the general population of Puerto Rico. This is further emphasized in figure 6 and table 4, which compare the tuberculin sensitivity of La Perla children with that of the general population of Puerto Rico and of other areas throughout the world (8).

These results suggest that figures on general population groups may not always represent actual conditions in certain slum areas. It is possible that similar conditions exist in other parts of the world and that the effectiveness of tuberculosis case-finding programs might increase by means of house-to-house testing in slum areas. This may be of greater importance in countries with high-prevalence rates of tuberculosis where large reservoirs of the disease might be encountered in slums. Slum surveys may gain importance, if, in the future, antimicrobial therapy with isoniazid becomes the method of choice in managing the inital tuberculous infection.

TABLE 4

PERCENTAGES OF TUBERCULIN-POSITIVE REACTORS ACCORDING TO AGE IN VARIOUS COUNTRIES*

Country	Period	Type of Tuberculin Test	Age				
			1 year	2 years	3 years	4 years	5 years
			per cent	*per cent*	*per cent*	*per cent*	*per cent*
Puerto Rico (slum of La Perla)	1955	PPD-S (0.0001 mg./0.1 ml.)	10.4	25.9	56.5	73.8	88.9
Puerto Rico (general population)	1951	PPD-S (0.0001 mg./0.1 ml.)	3.7	6.1	11.1		17.1
Tunisia	1949–50	Moro†	7.3	10.0	13.6	17.9	23.7
Poland	1948–49	Moro	8.2	9.6	12.5	16.5	21.2
Israel	1949–50	Moro	6.0	7.4	9.6	10.0	10.8
Greece	1948–50	Moro	2.6	3.6	4.8	6.2	7.6
Malta	1950	Adrenalin-Pirquet	1.7	2.6	2.9	4.4	5.6

* Figures of Tunisia, Poland, Israel, Greece, and Malta were obtained from the reports of the International Tuberculosis Campaign, "Mass BCG vaccination campaigns, 1948–1951." Figures of the general population of Puerto Rico were taken from the reports on the BCG vaccination program.

† The tuberculin jelly for the Moro patch tests was prepared from OT to which a sufficient quantity of PPD had been added to make the preparation 3 times stronger than the international standard OT.

SUMMARY

A tuberculosis case-finding survey among children less than five years of age, performed in a slum area in Puerto Rico, revealed a very high incidence of positive tuberculin reactors (35.8 per cent) as compared with figures available on the general population of Puerto Rico and other areas of the world. The method of conducting the survey included house-to-house tuberculin testing. The entire population of the slum area less than five years of age was tuberculin tested. The reaction was observed in 475 children; 170 showed positive reactions. Roentgenographic evidence of tuberculosis was discovered in 54.3 per cent of the positive reactors less than two years of age, and in 59.0 per cent of those two to five years of age.

SUMARIO

La Tuberculosis Primaria en Lactantes y Niños Pequeños (Encuesta en Una Barriada Pobre de Puerto Rico)

Una encuesta de descubrimiento de casos de tuberculosis en niños de menos de cinco años de edad, ejecutada en un barrio pobre de Puerto Rico, reveló una incidencia muy alta (35.8 por ciento) de reactores a la tuberculina, comparada con las cifras disponibles para la población general de Puerto Rico y para otras zonas del mundo. El sistema seguido en la encuesta comprendió comprobación con tuberculina de casa en casa, haciéndose esto en todas las personas de menos de cinco años de edad de la zona en cuestión. Se observó el resultado de la prueba en 475 niños; 170 revelaron reacciones positivas. Se descubrieron

signos radiográficos de tuberculosis en 54.3 por ciento de los reactores de menos de dos años de edad y en 59.0 por ciento de los de dos a cinco años de edad.

Resume

Primo-infection tuberculeuse chez les nourrissons et les jeunes enfants (Enquête dans une zone populeuse de Porto-Rico)

Une enquête de dépistage de la tuberculose chez les enfants âgés de moins de cinq ans, effectuée dans une zone populeuse de Porto-Rico, a révélé une très haute fréquence de sujets à tuberculine-test positif (35,8%) par comparaison aux chiffres obtenus parmi la population générale de Porto-Rico et d'autres régions du monde. La méthode employée dans cette investigation consistait à pratiquer le test à la tuberculine successivement dans chaque foyer après l'autre. La totalité de la population âgée de moins de cinq ans a été testée à la tuberculine. La réaction a été observée chez 475 enfants, 170 avaient une réaction positive. Les signes radiologiques de la tuberculose furent décelés chez 54,3% des sujets "positifs" âgés de moins de deux ans et chez 59% de ceux âgés de deux à cinq ans.

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THE LONG-TERM EFFECTS OF EXPOSURE TO NITROUS FUMES[1]

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INTRODUCTION

Exposure to nitrous fumes may result in several types of clinical reaction. One of these, acute pulmonary edema (1–5), develops after a latent period (lasting in some cases up to thirty hours), is frequently fatal, but is apparently followed by complete recovery if the subject survives the acute initial episode. Nevertheless, there are records of patients in whom symptoms persist. For instance, in Lindquist's sixteen cases, the patient of one case, on resuming work four months after the accident, found that he became breathless very easily, a complaint he had not noticed before his accident (5). Similarly, Johnstone (6), in his textbook, states that permanent disability occurs but is rare. However, no detailed follow-up studies by clinical methods or by pulmonary function tests have been reported, and there is no clear description of the nature of the residual disability or of the frequency of its occurrence.

A second type of reaction to these fumes is bronchiolitis obliterans (4, 7, 8). This condition can only be diagnosed with certainty by histologic methods, although the clinical course may suggest the diagnosis. Thus, exposure and acute symptoms are followed by a latent period, apparent improvement sometimes lasting up to a month (4, 5, 7–9). Thereafter there is progressive development of unremitting dyspnea with severe cough and frank cyanosis and typical roentgenographic findings of irregular soft mottling throughout both lungs. If the patient recovers, these roentgenographic changes clear surprisingly quickly. A third clinical picture following exposure to these fumes is that of pneumonia, apparently chemical rather than bacterial (10, 11). However, no work on the incidence and nature of permanent sequelae following bronchiolitis obliterans or on chemical pneumonia following exposure to nitrous fumes was encountered.

From the medical literature it was not possible to gain any clear impression as to whether permanent disability occurs after exposure to nitrous fumes. In view of this uncertainty, the present writers have made a long-term study of 7 patients who recovered from an episode of acute pulmonary edema following exposure to nitrous fumes in an attempt to see if residual disturbances of lung function could be demonstrated and, if so, to determine the pattern and degree of such disturbances. It was thought that the use of objective tests would be particularly valuable because exposure to gas in most instances is an industrial accident in which there is a possibility of compensation for disability. The victim is, therefore, perhaps less likely to be as objective as he might be about his subsequent complaints.

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TABLE 1

SUMMARY OF CLINICAL DATA

Number	Age	Height	Weight	Period of Underground Employment	Duration of Exposure to Fumes	Delay Before Development of Pulmonary Edema	Time Away from Work	Grade of Dyspnea*	
								Before	After†
	years	*cm.*	*kg.*	*years*	*minutes*	*hours*	*days*		
1	33	174	74	12	20	9	248	0	3
2	35	182	82	2	75	5	106	0	0
3	21	173	67	2	5	26	17	0	0
4	20	170	66	2	30	3	120	0	3
5	31	183	84	7	Unknown	5	70	0	2
6	49	183	100	13	30	27	102	0	3
7	39	191	102	—	10	14	17	0	3

* After Gilson and Hugh-Jones (17).

† At most recent examination.

MATERIAL AND METHODS

In the 7 cases studied, 6 of the patients were gold miners whose underground service is recorded in table 1. In *Cases 1, 3, 4, 5,* and *6,* exposure to nitrous fumes occurred following a blasting operation underground in which ammonium dynamite had been used. The patient of *Case 2,* employed in a uranium reduction plant at the time of the accident, inhaled acrid brown fumes resulting from the interaction of nitric acid and wood blocks. The patient of *Case 7* inhaled the toxic fumes in the course of cleaning a mixing vat in a dynamite factory in which he was employed. The exact composition and concentration of the constituents of the nitrous fumes are unknown. However, the work of Wade and associates (12) suggests that in ammonium dynamite explosion fumes there is a high percentage (approximately 52 per cent) of the more toxic nitrogen dioxide.

In each case there was a variable (three- to thirty-hour) latent period before the onset of respiratory symptoms. All patients except one[2] were seen in the state of acute pulmonary edema by one of the present writers (C. C. F.).

The clinical and roentgenographic features of pulmonary edema were typical, and in all cases the patient was desperately ill irrespective of the time of exposure and length of latent period between exposure and the onset of symptoms (table 1). In all cases except *Case 1,* steroid therapy was started promptly on the appearance of the chest signs, with early and good effect. *Case 1,* the first patient of this series to be seen, was handled by more conservative measures (atropine, oxygen, and antimicrobials) for five weeks during which time his condition deteriorated steadily. On the institution of corticotropin therapy, he started to improve dramatically, and this success led to the use of the same therapy in subsequent cases.

All patients claimed to have been quite healthy before the accident and all of them considered that their exercise tolerance was as good as, or better than, that of their colleagues of similar age.

The grade of dyspnea after the accident was assessed at the most recent follow-up examination (table 1).

Whenever possible, the first lung function study was carried out at the time of the patient's discharge from the hospital after recovery from the acute initial episode. Thereafter an attempt was made to repeat studies at yearly intervals.

Spirometry was performed on all subjects to measure vital capacity, expiratory reserve volume, and maximal breathing capacity. Functional residual capacity was measured by

[2] The patient of *Case 7* was diagnosed and treated by Dr. Dalton.

TABLE 2

RESULTS OF PULMONARY FUNCTION TESTS

Number	Time After Accident	Lung Volume Studies						Mixing Index	Oximetry†			Hugh-Jones Effort Test (17)		
		Vital Capacity	Per Cent of Predicted Value*	Residual Volume	Per Cent of Predicted Value*	FRC/TLC × 100	RV/TLC × 100		At Rest	On Exercise	On Oxygen	Resting Ventilation‡	Exercise (or Standard) Ventilation	Dyspneic Index
	months													
1	8	2.56	53§	1.93	116	69§	42§	5.66§	96	−4	99			
	55	3.48	74§	1.53	81	58	30	3.29	94	−3	102	12.0	48	69
	64	3.98	85§	1.74	92	51	30	3.35	94	−5	102	14.6	56.5	84
	65							2.74				10.9	44.4	52
2	1	3.8	72§	1.79	87§	47	32§	7.44§				15.7	40.5	45
	2	4.33	83§	1.77	86§	45	29	3.63§	89	−7		12.8	51.0	35
	6	4.81	92	1.93	94	47	28	2.92§	94	−5		9.5	32.4	21
	24	4.80	93	1.55	74§	40	24	2.32	92	−8	101	9.1	30.3	21
	37	4.80	93	2.41	114	40	33§	2.45	94	−2	100	8.6	40.5	22
3	1	5.09	100	1.69	106	61§	25	2.34	80	−2		12.8	32.2	23
	32	5.66	114	1.46	88	59	20	1.90	95	−4	100	9.4	33.8	18
4	3	3.81	77§	2.28	154§	52	36§	2.03	94	−4	102	10.5	34.2	45
	15	4.59	94	1.86	124§	49	28	1.95	88	−1	96	11.6	45.4	33
	26	4.48	92	1.41	107	63§	24	1.51	93	+1	100	9.6	55.6	37
5	1	5.52	102	2.02	100	54	27	3.59§	96	−8	—	12.2	34.8	27
	4	5.51	102	1.80	89	57	24	2.46	77	−10	—	14.7	34.6	23
	25	5.15	96	1.95	95	61	27	2.94	93	−9	102	11.5	40.8	29
6	1	5.39	110	2.54	109	44	32	1.77	93	−2	102	13.5	43.0	34
	7	3.14	64§	2.42	104	56	42§	2.47	87	−12	97	14.4	41.7	43
	18	4.86	101	3.22	137§	52	40§	1.88	94	−2	100	13.2	42.0	47
	19								96	−3	106	13.8	43.8	49
7	9	6.06	106	2.86	121§	62§	32	1.31	94	+1	101	11.8	41.6	31

* Reference (19).

† Arterial oxygen saturation.

‡ Ventilation in liters per minute, ambient pressure saturated with water vapor.

§ Results considered abnormal, i.e., differing from the predicted value by more than 2 SD of the prediction formulas (19).

the closed-circuit constant volume method of McMichael (13) with certain adaptations (14). From the rate at which the lungs equilibrated with this circuit, a mixing index was calculated (15, 16).

An exercise test of the type developed by Hugh-Jones (17) was performed by each patient, and the arterial oxygen saturation at rest, during this exercise, and after breathing pure oxygen was recorded by oximetry.[3] Finally, the mechanics of breathing were studied in each case by comparing intraesophageal pressure with rate of airflow in and out of the lungs. Intraesophageal pressure was measured with a tube of the type described by Mead and Whittenberger (18) and a straingauge manometer,[4] airflow using a Lilly pneumotachygraph screen. Compliance (expressed as liters per cm. water pressure) was estimated by re-

[3] Waters Conley Absolute Reading Oximeter.

[4] Statham P5 TC ± .7D-350.

TABLE 3

STUDIES OF THE MECHANICS OF BREATHING

Number	Time After Accident	Maximal Breathing Capacity*		Before Exercise				After Exercise			
		Liters/min.	Per cent Pred.†	Compliance‡	Resistance Inspiration	Resistance Expiration	Respiratory Rate	Compliance‡	Resistance Inspiration	Resistance Expiration	Respiratory Rate
	months										
1	8	71	44‖								
	55	68	43‖								
	64	85	54‖	0.173	1.84	4.04	26				
2	1	89	53‖								
	2	141	84‖								
	6	158	95								
	24	146	90‖								
	37	184	114	0.174	3.65	4.50	12	0.202	2.45	3.69	24
3	1	139	81‖								
	32	186	113	0.158	2.09	2.40	20	0.169	2.33	3.61	26
4	3	75	42‖								
	15	137	78‖								
	26	149	87‖	0.249	6.76	5.89	22	0.160	2.79	4.06	28
5	1	130	75‖								
	4	152	87‖								
	25	142	83‖	0.283	2.75	3.27	22	0.235	2.82	2.62	24
6	1	126	81‖								
	7	97	61‖								
	18	89	57‖								
	19			0.124	5.43	6.18	19	0.159	3.08	4.71	21
7	9	133	74‖	0.219	3.87	4.90	18	0.252	3.09	4.84	20
Mean value for 16 normal subjects				0.219	1.86	2.04	20				

* In liters per minute, ambient pressure saturated with water vapor.

† Percentage of predicted value (19).

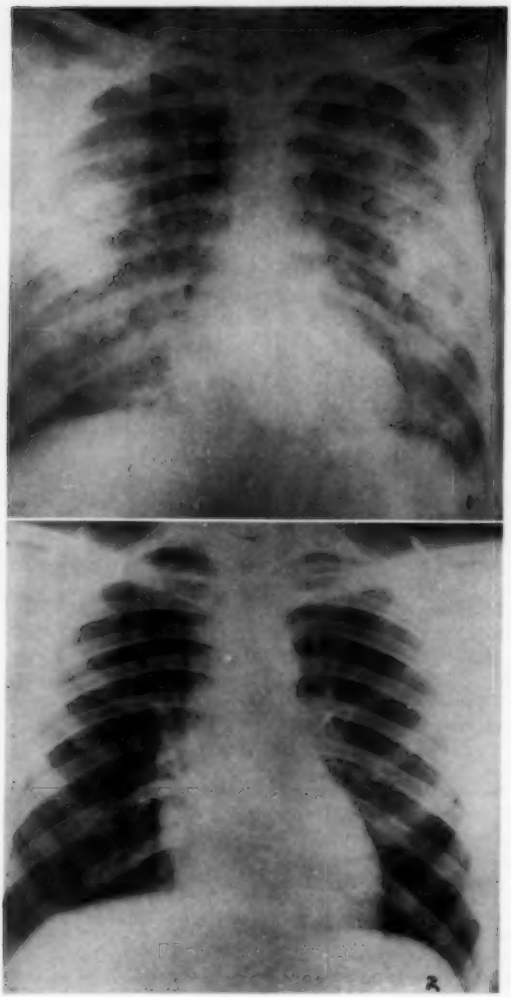
‡ Liters per cm. per water pressure.

§ Nonelastic resistance, cm/l/second.

‖ Differs from predicted value significantly.

lating tidal volume to the intrathoracic pressure difference at end-inspiration and end-expiration, and resistance (expressed as cm. per liter per sec.) was calculated at the points during inspiration and expiration at which the pressure required to overcome nonelastic resistance was maximal (18).

Certain results were compared with the predicted normal value for the particular individual, namely VC, RV, $\frac{FRC}{TLC} \times 100$, $\frac{RV}{TLC} \times 100$, distribution index (19). The prediction formulas used were based on a study of 94 normal subjects of various ages, resident at the altitude of this locality (5,670 feet above sea level), in which test results were correlated with four body characteristics (namely age, height, weight, and surface area), and formulas for prediction were based on one or more of these variables by standard statistical techniques of partial correlation. The accepted limits of accuracy in prediction were those bounded by twice the standard deviation of the prediction formula.



Case 4

FIGS. 1 and 2: The patient had to climb an incline of about 400 feet through fumes to reach a fresh-air stope underground. He was taken to the hospital and within three hours signs of pulmonary edema (including cyanosis, breathlessness, and scattered rhonchi and rales) had appeared. Corticotropin and oxygen were administered followed by oral cortisone.

Fig. 1. Chest roentgenogram, obtained at the bedside twenty-four hours after the accident, shows extensive opacities throughout both lung fields and surgical emphysema.

Fig. 2. Roentgenogram, obtained nineteen days later when the patient was clinically recovered and ready to be discharged from the hospital, shows normal lung fields.

Results

The results of the study are summarized in tables 1–3. Four patients (*Cases 1, 4, 6,* and *7*) complained that following the accident their capacity for exertion was impaired to the extent that they could no longer keep up with their colleagues when walking on the level, although they could probably manage to walk a mile at their own speed on flat ground. In all of these cases expiratory resistance values were high and maximal breathing capacity figures were reduced. In addition, 2 patients (*Cases 6* and *7*) showed an increase in the absolute value for residual volume, suggesting overinflation of the lungs at rest. One patient (*Case 5*) complained of mild dyspnea following the accident. His pulmonary function tests were within normal limits except for slight reduction in maximal breathing capacity and expiratory resistance values at the upper limits of normal or slightly above normal.

Two patients only (*Cases 2* and *3*) considered that their capacity for effort was unaffected by the accident. Pulmonary function studies in one (*Case 3*), even as soon as a month after the accident, were normal except for a slightly reduced maximal breathing capacity, and thirty-two months after the accident all tests performed were normal.

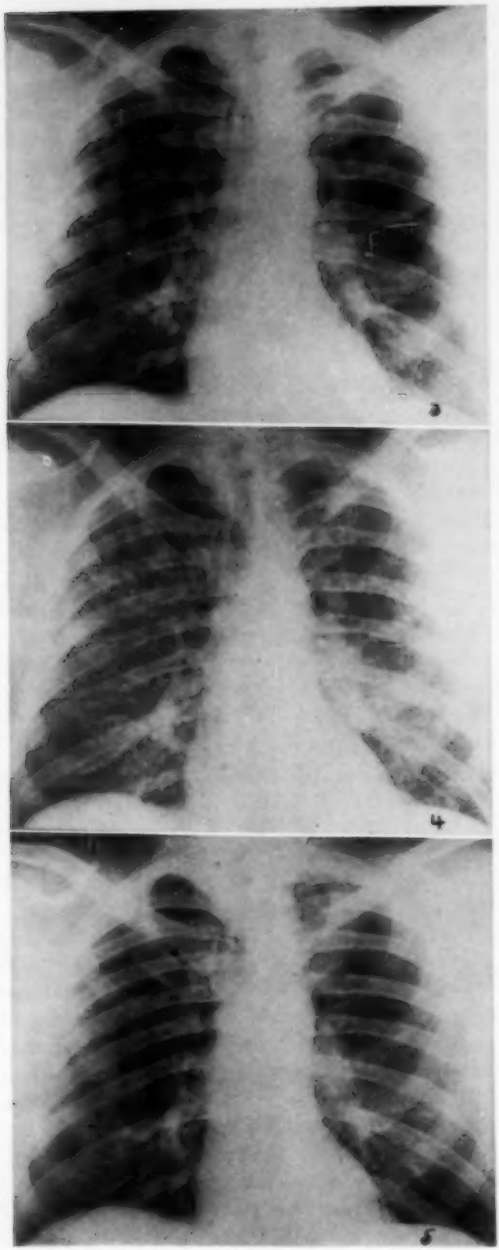
In the other patient (*Case 2*), test results returned to normal somewhat more slowly. The most recent studies thirty-seven months after the accident again show normal values for all tests except for an increased nonelastic resistance. The inconsistency of a high breathing resistance but normal breathing capacity could perhaps be the result of good muscle power (20), a factor which was not assessed in these cases. In *Case 2* (an amateur weight-lifter), it is at least possible that physical training had developed the thoracic muscles to a degree above average and that for this reason the patient was able to overcome the increased resistance without being conscious of the greater effort necessary.

Chest roentgenograms of 2 of the patients (*Cases 4* and *6*) are included in this report.

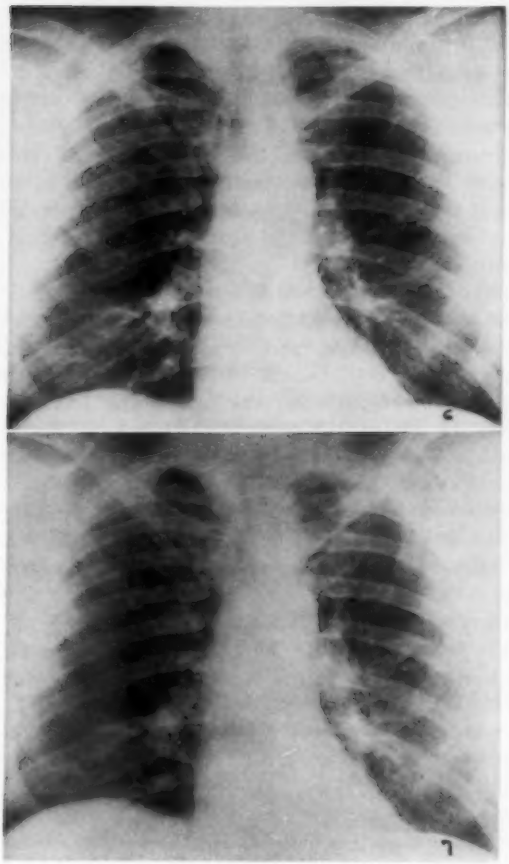
Discussion

One of the primary aims of the present study was to discover whether disturbances of lung function could be demonstrated to account for persistent symptoms reported by 5 of 7 subjects who had survived an episode of acute pulmonary edema following exposure to nitrous fumes. In all of the 5 patients who complained of symptoms (*Cases 1, 4, 5, 6,* and *7*) similar disturbances of function were noted, namely, a decrease in maximal breathing capacity and an increase in the nonelastic work of breathing. In *Case 5*, the values for nonelastic resistance were only very slightly raised, a finding in accordance with the patient's complaint of only mild symptoms. Of the 2 patients who claimed to have recovered completely (*Cases 2* and *3*), all test values were normal in one instance (*Case 3*) while in *Case 2* high nonelastic resistance values were found, but the value for maximal breathing capacity was normal. Thus, a good correlation has been found between symptoms and disturbances of lung function test.

The mining service of the first 6 patients is important in that it was necessary



FIGS. 3-5



Case 6

FIGS. 3–7: Serial chest roentgenograms of the patient of *Case 6*. Following exposure to blasting fumes underground, the patient was placed under observation in the hospital for twelve hours and then discharged. About fifteen hours later, i.e., twenty-seven hours after the accident, he developed tightness of the chest and gurgling behind the sternum. On readmission to the hospital, pulmonary edema was diagnosed and steroid therapy was started.

Fig. 3. Chest roentgenogram, obtained a year before the accident, shows normal lung fields, with no signs of pneumoconiosis.

Fig. 4. Chest roentgenogram, obtained twenty-four hours after the accident, shows the appearance of pulmonary edema.

Figs. 5, 6, and 7. Chest roentgenograms, obtained seventy-two hours, eighteen months, and twenty-six months, respectively, after the accident show rapid clearing of the shadows, with no residual changes.

to be certain that none of the functional changes observed could be ascribed to early stages of pneumoconiosis. In 3 cases the period of employment in a dusty occupation was insignificant (*Cases 2, 3,* and *4*). In *Cases 1, 5,* and *6,* service was for periods of six years and more, and might, according to the figures available (21), have resulted in a dust disease of the lung. In the 6 miners it was possible to study roentgenograms obtained at the Silicosis Medical Bureau at the time of the compulsory annual medical examination. In each instance the roentgenogram taken at the examination preceding the accident appeared normal, and it seemed unlikely, therefore, that the disturbances of function observed were the result of pneumoconiosis.

The type of physiologic disturbance demonstrated was interesting. Impairment was noted in the tests most intimately concerned with free airflow in and out of the lung, i.e., maximal breathing capacity and nonelastic breathing resistance. Common causes of such disturbances include bronchial obstruction (due to spasm or secretion) and emphysema, when a check-valve mechanism has been suggested (22, 23).

In the cases described in this report, there was no evidence to suggest that bronchospasm was a factor which might account for the persistent clinical symptoms. Measurements of maximal breathing capacity before and after the administration of an antispasmodic drug (neoepinine,® 5 mg. sublingually) were similar; there was no clinical history of spasmodic dyspnea, and rhonchi were not recorded on any of the physical examinations once the patient had recovered from the episode of acute pulmonary edema.

Similarly, there was no reason to think that the development of emphysema subsequent to the acute episode could account for the persistent symptoms. Roentgenographic changes did not suggest such a development, nor were there changes in the test results to support this pathology. It should be noted that, in 2 patients in whom symptoms persisted (*Cases 6 and 7*), high values were recorded for residual volume; but in neither of these patients was intrapulmonary mixing disturbed, nor was there any significant fall of arterial saturation noted on effort to indicate a defect in diffusion. In the absence of such disturbances, emphysema of a significant degree is unlikely (24).

Consideration should be given to the possibility that the high nonelastic resistance values observed might be the result of an increase in tissue resistance, either instead of, or as well as, an increase in airway resistance. Unfortunately, it was not possible to distinguish these two components of nonelastic resistance by the techniques used in this study.

A possible explanation for the high resistance values occurred to the present writers after reading McAdams' description of a case of bronchiolitis obliterans following exposure to nitrous fumes (9). Narrowing of bronchial passages would provide a likely explanation for the physiologic defects observed in the present cases, and a possible pathologic cause for this narrowing might be the residua of some degree of bronchiolitis obliterans in which the disease process had healed by fibrosis, leaving many airways partially occluded. Consideration has been given to the fact that none of the present cases followed the apparently typical

clinical course of bronchiolitis obliterans, namely, a delay of four to six weeks after initial exposure to the gas before an acute clinical illness developed (1, 9). It is possible, however, that the steroid therapy had influenced the pattern of the disease in these cases. In addition, animal studies (25) as well as various post-mortem studies on man suggest that the pathologic picture may be mixed (11, 27). It seemed reasonable, therefore, to suppose that some bronchioles had developed an obliterative condition although the main pathologic processes in the lung had been those of acute chemical pulmonary edema.

Summary

Seven patients who had recovered from an episode of acute pulmonary edema following exposure to nitrous fumes were studied for periods up to sixty-four months after the accident. There was fairly good agreement between subjective complaints and demonstrable disturbances of physiologic tests.

The usual pattern of physiologic disturbance was reduction in maximal breathing capacity and increase in expiratory resistance values. Two patients showed an increase in the absolute value of residual volume as well as an increase in its percentage value of total lung capacity. Such changes in the test values are commonly associated with the presence of emphysema or bronchospasm. Neither of these conditions was thought to be present in the reported cases. A possible explanation seemed to be that the patients had some degree of bronchial and bronchiolar narrowing due to the healing by fibrosis of various degrees of bronchiolitis obliterans following exposure to nitrous fumes.

Sumario

Los Efectos a Largo Plazo de la Exposición a los Vapores Nitrosos

Siete enfermos que se habían repuesto de un episodio de edema pulmonar agudo consecutivo a exposición a vapores nitrosos fueron estudiados durante periodos que alcanzaron hasta sesenta y cuatro meses después del accidente. Hubo bastante buen acuerdo entre los síntomas subjetivos y los trastornos observables en las pruebas fisiológicas.

El patrón habitual del trastorno fisiológico fué reducción de la capacidad respiratoria máxima y aumento de los valores de la resistencia espiratoria. Dos enfermos mostraron aumento del valor absoluto del volumen residual así como aumento del valor porcentario de la capacidad pulmonar total. Esas alteraciones en los valores de ensayo se vinculan comúnmente con la existencia de enfisema o broncospasmo. No parecía que hubiera ni una u otra de esas dolencias en los casos discutidos. Una posible explicación parecía ser que los enfermos tenían alguna estenosis bronquial y bronquiolar debida a cicatrización por fibrosis de una bronquiolitis obliterante de intensidad variable, dolencia esta que se ha descrito a continuación de la exposición a vapores nitrosos.

Resume

Les effets éloignés de l'exposition aux vapeurs nitreuses

Sept malades ayant survécu à une crise d'oèdeme pulmonaire aigu succédant à l'exposition aux vapeurs nitreuses ont été étudiés pendant des périodes allant jusqu'à soixante-quatre mois après l'accident. La concordance entre les symptômes dont se plaignaient les malades et l'altération des tests physiologiques était relativement bonne.

Les modalités habituelles des troubles physiologiques consistaient en une réduction de la capacité de ventilation maxima et un accroissement des valeurs de la résistance ex-

piratoire. Deux sujets présentaient une augmentation de la valeur absolue du volume résiduel et une augmentation de la valeur de son pourcentage par rapport à la capacité pulmonaire totale. Ces variations des valeurs observées dans les tests sont ordinairement associées à l'emphysème et au bronchospasme. Ni l'une ni l'autre de ces affections n'était considérée présente dans les cas rapportés. L'explication plausible paraissait être l'existence, chez ces malades, d'un certain degré de rétrécissement bronchique et bronchiolaire dû à un processus de sclérose cicatricielle à la suite de différents degrés de bronchiolite oblitérante, phénomène ayant été décrit à la suite de l'exposition aux vapeurs nitreuses.

Acknowledgments

The writers acknowledge with gratitude the help of Professor G. A. Elliott, in whose department the physiologic studies were carried out. They are also grateful to Dr. Goedvolk of the Chamber of Mines Hospital for permission to publish these cases, and to Dr. M. Weinbren for allowing them to reproduce roentgenograms taken in his department. Dr. G. Dalton of the Modderfontein Dynamite Factory kindly allowed them to study the patient of *Case 7*. The use of steroid therapy was originally suggested by Dr. M. M. Suzman who saw the patient of *Case 1* in consultation.

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HEPATIC DERANGEMENT IN PULMONARY TUBERCULOSIS[1, 2]

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INTRODUCTION

It has been a frequent observation that pulmonary tuberculosis is found to be associated with deranged liver function tests. Surprisingly enough, the hepatic damage has received very little attention. No doubt the great reserve power of the liver is responsible for the neglect of this aspect. Although clinical evidence points to a lack of significant hepatic parenchymal damage in patients with pulmonary tuberculosis, sensitive liver function tests together with biopsy studies indicate otherwise.

Haex and Van Beek (1) claimed that they could diagnose very early cases of pulmonary tuberculosis by histopathologic examination of the liver. This might seem to be a sweeping and bold statement. However, undoubtedly these observers have done the most extensive work in this field; they examined hundreds of serial sections of biopsy material. Haex and Van Beek's studies led to the present investigation.

Moreover, it seemed advisable to establish correlation between the clinical, roentgenographic, and bacteriologic examination, on one hand, with the hepatic derangement, on the other hand, as evaluated by morphology and sensitive liver function tests. This aspect of the problem is still quite untouched. The present investigation incorporates a critical study of 100 cases of pulmonary tuberculosis in which liver function tests were carried out along with the biopsy studies.

MATERIAL AND METHODS

The present series includes 100 cases of pulmonary tuberculosis, confirmed by clinical, roentgenographic, and bacteriologic procedures. The patients were admitted to the medical wards of Sawai Man Singh Medical College Hospital. The criterion of admission to the clinic was pulmonary infiltration although, of 100 cases studied, there were 4 cases of pleurisy with effusion which appeared to be tuberculous. This was confirmed in 2 cases by culture positive for tubercle bacilli; there were 2 cases of tuberculous lymphadenitis.

On the day of admission, the routine history, physical examination, blood counts, erythrocyte sedimentation rate, bleeding time, and coagulation time were done. On the second day the patients were screened to discover the actual extent and character of the pulmonary lesion; if needed, skiagrams were taken. Later, the following liver function tests were done: thymol turbidity, total plasma proteins, albumin-globulin ratio, vandenberg (qualitative), icteric index, alkaline phosphatase, serum cholesterol, and colloidal gold flocculation. Prothrombin time was done with "thrombokinase tablets, Geigy," by modified Quick's method.

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[2] Portions of the present study are to be submitted by Dr. R. K. Bhargava to the University of Rajputana in partial fulfillment of the requirements for the degree of Doctor of Medicine.

On the same day, sputum, stool, and urine examinations were performed, the latter chiefly for urinary urobilinogen. In all cases serologic tests for syphilis (Kahn) were done.

On the third day, liver biopsy was done with Vim Silvermann's needle, after the administration of some rapidly acting barbiturate.

Patients were asked to take complete rest in bed for twenty-four hours after biopsy, during which time the pulse was recorded hourly. On the fourth day sputum was re-examined microscopically three or four times and also by a concentration method. In 4 cases of pleural effusion, the pleural fluid was cultured for acid-fast bacilli on Löwenstein-Jensen medium. If the sputum was found repeatedly negative for acid-fast bacilli even after concentration methods, then a tuberculin test (Mantoux) was done with PPD tuberculin, 1:10,000.

In some patients with sputum negative for *M. tuberculosis*, a biopsy from axillary or cervical lymph nodes was performed. Some special tests were also done to exclude other diseases which can produce lesions similar to those seen in tuberculosis, e.g., the Brucella agglutination test, the Paul-Bunnel test, and the lepromin test. As a routine procedure, liver biopsy specimens were fixed in 90 per cent alcohol and stained with hematoxylin and eosin. Reticulum and glycogen stains were also done routinely. In addition, for those patients who showed definite granulomatous lesions, the sections were also stained for acid-fast bacilli by a modified Ziehl-Neelsen method.

Observations

The extent of the pulmonary tuberculosis was classified according to the *Diagnostic Standards*, published by the National Tuberculosis Association, 1950 (United States) into: far advanced lesions, moderately advanced lesions, and minimal lesions. Ninety-one of 100 cases were in the above three categories. In addition, there were 3 cases of miliary tuberculosis, one case of pulmonary infiltration with pleural effusion, 4 cases of mere pleural effusion, and one case of diffuse reticulation in both lung fields. In 80 of these patients, sputum was positive for acid-fast bacilli. The remaining 20 patients whose sputum was negative for acid-fast bacilli had positive Mantoux tests with 1:10,000 PPD, 8 had lymph node biopsies positive for *M. tuberculosis*, and 2 had sputum cultures positive for tubercle bacilli. Thus, in all there were 10 cases in which no bacteriologic diagnosis could be made.

There were 55 cases of pulmonary tuberculosis, in which abdominal signs or symptoms of one kind or another were present. The symptoms commonly observed were anorexia, pain, feeling of distension, constipation, and diarrhea. Hepatic enlargement was present in 33 cases. Other positive findings were splenic enlargement in 8 cases; a nodular lump in the abdomen in 3; palpable and gurgling cecum in 3; palpable and tender colon in 12; diffuse abdominal tenderness in 3; and the presence of free fluid in 7 cases.

The derangement in liver functions and changes in the morphology are represented in figures 1 to 3.

In figure 1, the alteration in the liver function tests is presented. Twelve liver function tests were done in each case. A single vertical line represents one specific liver function test and is made up of 100 small squares, so as to show the derangement in that particular liver function test in the 100 cases studied. The criteria of derangement as slight, moderate, and marked are based on arbitrary figures as adopted by Popper and associates (2) although in certain tests, e.g., total

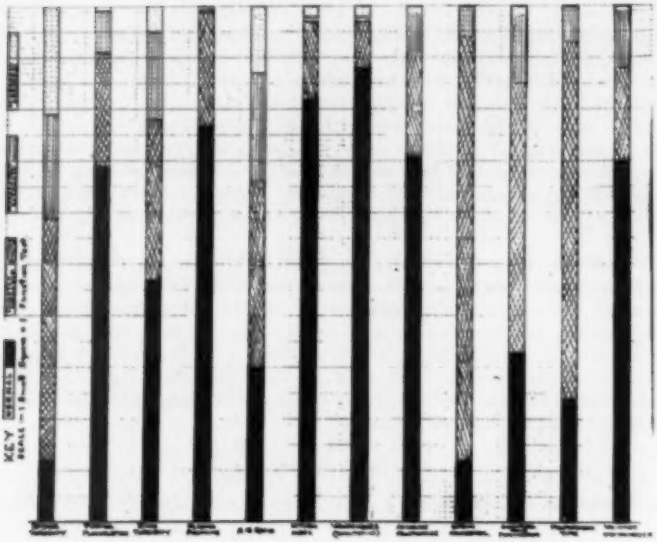


FIG. 1. Graph showing derangement in liver functions.

serum cholesterol, gradations had to be adopted which were based on the writers' observations with these tests and are not necessarily the accepted standards. (In table 1 the criteria may be seen for the derangement of the liver function tests as observed in the present study.) In all of these tests, although thymol turbidity, serum cholesterol, prothrombin time, and albumin-globulin ratio were found to be altered in most of the cases, the colloidal gold flocculation test was found to be the best guide, for it was most satisfactorily correlated with both the clinical and morphologic findings. The colloidal gold test in the more advanced lesions showed more of the flocculation and was positive more frequently than the other tests. Urinary urobilinogen estimation also deserves separate mention, for in some cases it was found to be positive at the time when other liver function tests were within normal limits. The other tests performed did not throw much light on the extent of the derangement, both clinical and morphologic.

The morphologic changes found in the series are demonstrated in figure 2. The most important features in morphology were the tubercles of submiliary size found in the liver on microscopic examination. The tubercles were classified into four categories. The first group showed caseation with giant cells, epithelioid cells, mononuclears, and fibroblasts (figure 3). The second group resembled the first except that the caseation was absent (figure 4). The third group consisted of epithelioid cells and lymphocytes but no giant cells (figure 5). The fourth group showed only marked lymphocytic and histiocytic infiltration in the periportal spaces or intralobularly with the surrounding fibroblasts (figure 6). The most interesting of these were the first and the second groups which together included 10 of the 100 cases. The tubercles were seen chiefly in the periportal region.

TABLE 1

CRITERIA FOR THE DERANGEMENT OF THE LIVER FUNCTION TESTS

Range	Liver Function Tests											
	Thymol Turbidity	Thymol Flocculation	Zinc Turbidity	Total Plasma Proteins	Albumin Globulin Ratio	Colloidal Gold Flocculation	Icteric Index	Vandenbergh (Qualitative)	Total Serum Cholesterol	Alkaline Phosphatase	Prothrombin Time	Urinary Urobilinogen (Qualitative)
	units		*units*	*gm.*			*units*		*mg.*	*units*	*per cent*	
Within normal limits	0–4	1+	0–8	>5	2:1	0 to 1+	0–7	Negative	>140	0 to 10	85 or more	Present in 1:10 dilution
Slightly deranged	5–6	2+	9–12	4–5	3:2	2+	8–14	Indirect positive	100 to 140	11 to 15	71 to 84	Present in 1:20 dilution
Moderately deranged	7–10	3+	13–16	3–4	1:1	3+	15–50	Delayed direct positive	<100	16 to 20	50 to 70	Present in 1:30 dilution
Markedly deranged	>10	4+	>16	<3	2:3 or 1:2	4 to 5+	>50	Immediate direct positive	<75	>20	<50	More than 1:30 dilution

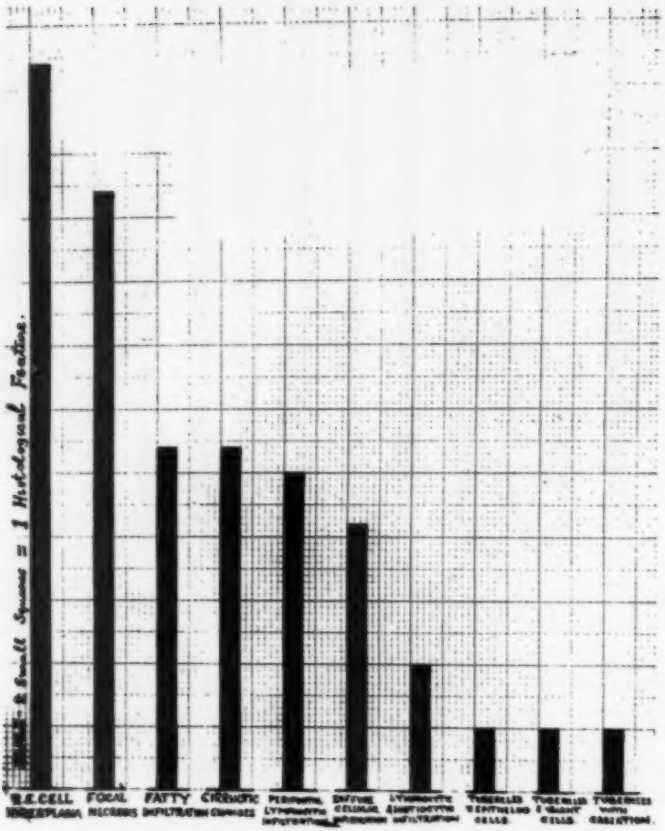


FIG. 2. Graph showing histopathology of liver.

In some of the 10 cases acid-fast bacilli were also demonstrated in the tissues. It was observed that caseation need not be present, for the tubercles without caseation revealed acid-fast bacilli, while the tubercles with caseation resembled sarcoidosis, at least in 2 cases of the series.

The third and the fourth groups comprised 15 of the 100 cases. These consisted of granulomas either with epithelioid cells or with lymphocytic and histiocytic cellular infiltration. These lesions were believed to be highly suggestive of the tuberculous involvement of the liver for the following reasons: (*1*) These lesions when studied by serial sections showed giant cells. (*2*) The possibilities of other granulomas, such as syphilis, brucellosis, sarcoidosis, leprosy, or mycotic infection, were excluded by suitable tests. (*3*) Definite tuberculous involvement of lungs was present in 90 per cent of these cases.

Reticuloendothelial cell hyperplasia was the common feature observed (figure

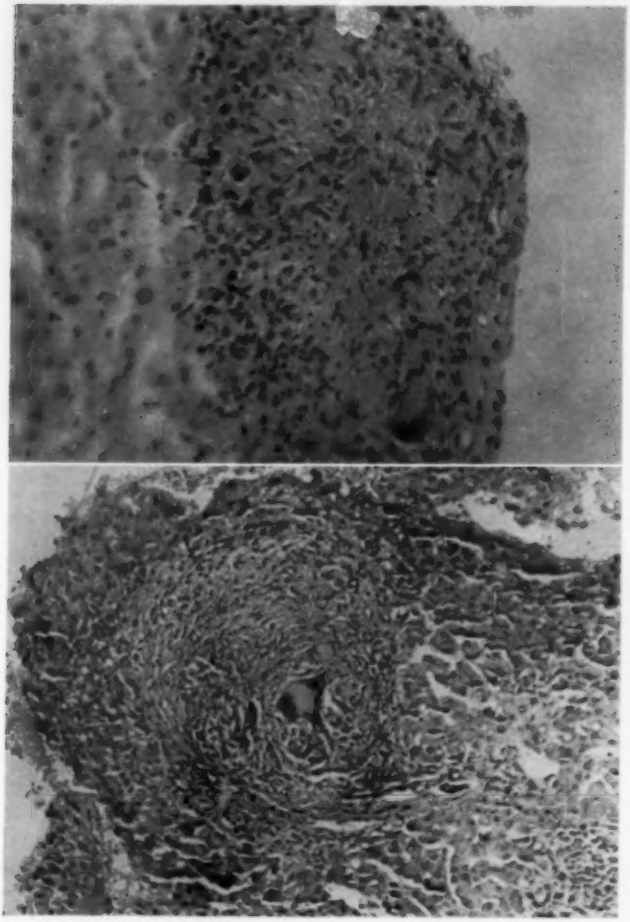
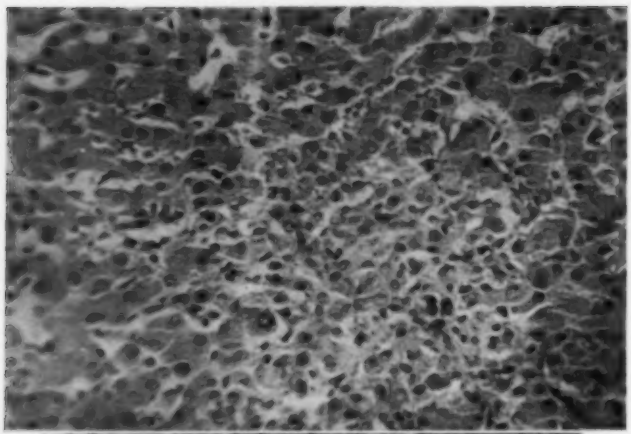


FIG. 3 (Upper). Photomicrograph of a single tubercle at one end of the biopsy material showing early central caseation with epithelioid cells, giant cells, and lymphocytes. (H. and E. stain × 270)

FIG. 4 (Lower). Photomicrograph of another single tubercle consisting of epithelioid cells and two giant cells from a patient with pleural effusion. (H. and E. stain × 270)

7). It was marked in 3 cases and moderate in 54 cases. Focal necrosis too was quite frequent (figure 8), being marked in 3 cases and moderate in 44 cases. Fatty infiltration was next in frequency, being slight in 16 cases, moderate in 6 cases, and marked (with fatty cysts) in 5 cases (figure 9). In addition, moderate periportal lymphocytic infiltration was present in 25 cases (figure 10), while mild periportal or sinusoidal lymphocytic infiltration was present in 21 cases. A moderate



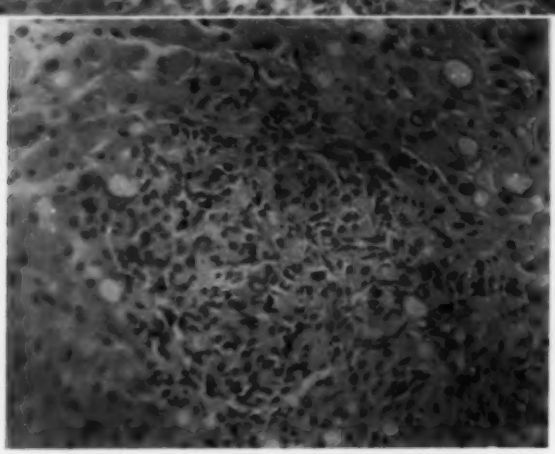


FIG. 5 (Upper). Photomicrograph of an epithelioid tubercle with few lymphocytes seen in the periportal region. (H. and E. stain × 270)

FIG. 6 (Lower). Photomicrograph of a focal collection of histiocytes and lymphocytes in one of the lobules of the liver of a patient with pulmonary tuberculosis. (H. and E. stain × 270)

increase in the quantity and density of periportal connective tissue was found in 16 cases, while a marked increase was found in 11 cases. As would be expected in chronic granulomatous lesions, there was marked increase of reticulum fibers which were well condensed in the granulomatous nodules.

Other changes not shown in figure 2 were: sinusoidal dilatation in 14 patients; disorganization of the liver plates in 6; cloudy swelling in 5; golden yellow pigment in liver cells in one; bile pigments in the liver cell and reticuloendothelial cells in 3; vacuolization of the liver cells in 6; bile thrombi with obstructive jaundice in one; serous hepatitis in one; passive congestion in 4; phlebosclerosis

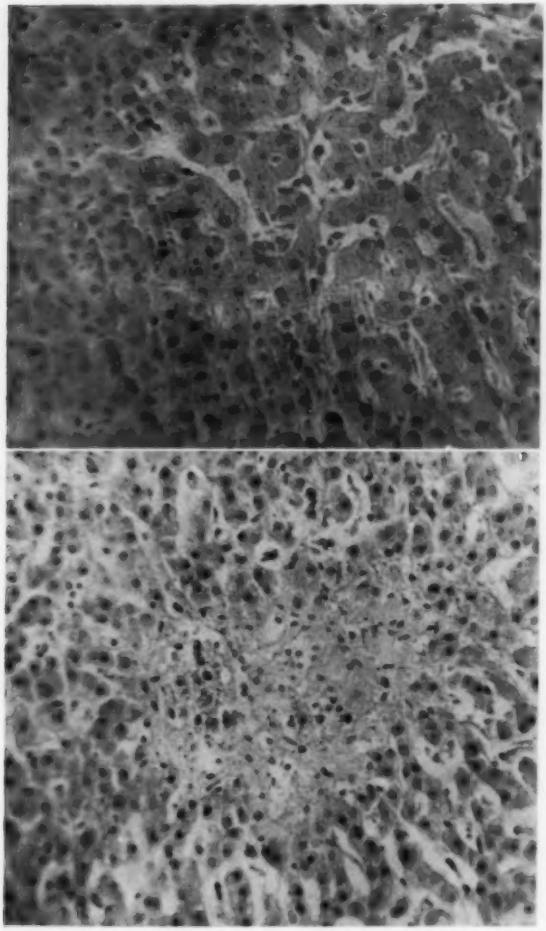


FIG. 7 (Upper). Photomicrograph showing reticuloendothelial hyperplasia in which proliferating Kupffer's cells are seen inside the sinusoids. (H. and E. stain × 270)

FIG. 8 (Lower). Photomicrograph of focal necrosis of liver showing liver cell fragments with little cellular reaction in a patient with pulmonary tuberculosis. (H. and E. stain × 270)

of the central vein in one; regenerating nodules in 2; and bile duct proliferation in 2 patients.

In figure 11, the clinicopathologic correlationship is presented. For this purpose the cases studied were divided in four categories: advanced, moderate, early, and miscellaneous, based on roentgenographic findings. The miscellaneous group included cases of miliary tuberculosis, pleural effusion, and one with old fibrotic

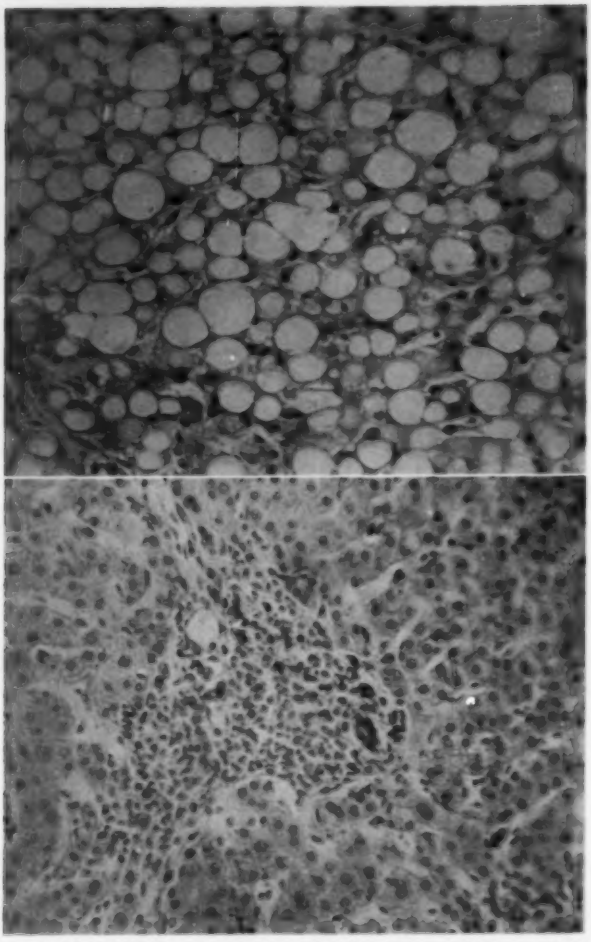


FIG. 9 (Upper). Photomicrograph showing extensive amount of fatty infiltration of the liver with a prominent fatty cyst in the center in a patient with pulmonary tuberculosis. (H. and E. stain × 450)

FIG. 10 (Lower). Photomicrograph showing a nodular collection of lymphocytes in the periportal region. Early periportal fibrosis is also present. (H. and E. stain × 270)

strands in the lung fields. These groups were further arbitrarily subdivided on the basis of the number of acid-fast bacilli found in the sputum, as shown in table 2. The roentgenographic and bacteriologic studies were correlated with clinical hepatomegaly, derangement in the liver function tests, and alteration in the liver morphology. The figure consists of 100 horizontal lines so that all of the features of an individual case may be studied by tracing a single horizontal line.

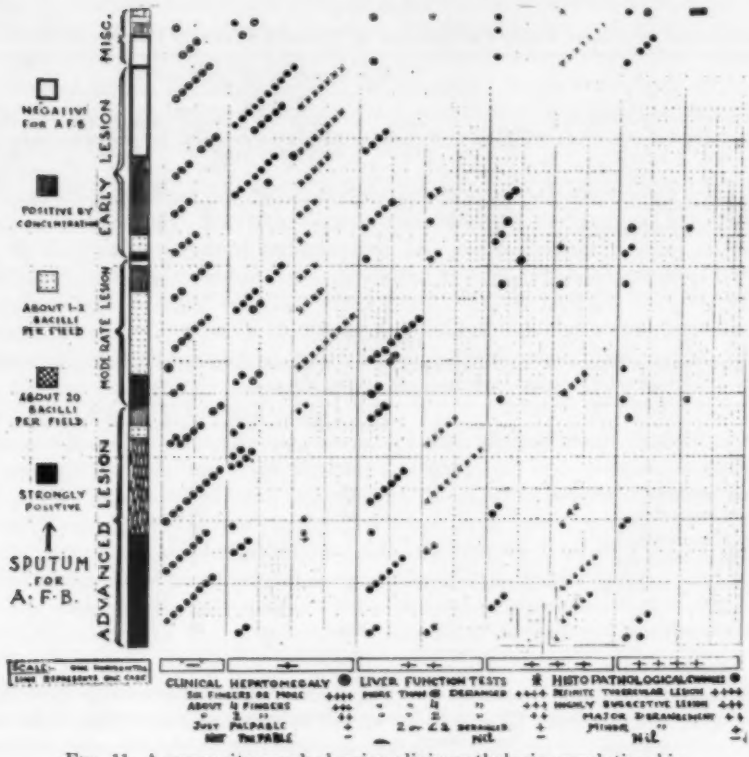


FIG. 11. A composite graph showing clinicopathologic correlationship.

The percentage of various morphologic changes in liver in cases of pulmonary tuberculosis are given in table 3, together with the number of cases in the present study compared with cases of other observers.

Clinopathologic Correlationship in the Present Study

Histopathology with hepatomegaly: Of 25 cases of definite or highly suggestive tuberculous lesions in the liver, hepatomegaly was found in 7 cases only. In the whole series hepatomegaly was seen in 33 cases. This shows that the presence of tuberculous lesions in the liver are not related to the clinical hepatomegaly.

Liver function tests with hepatomegaly: Fifty-six of 100 patients studied showed derangement of more than two functions of the liver. Of these, 23 patients had liver enlargement while the remaining 33 had no enlargement. Keeping in mind that there were 33 patients with hepatomegaly, there seems to be some correlation between hepatomegaly and derangement of liver function.

Liver function, hepatomegaly, and histopathology: When liver function, hepatomegaly, and histopathology were considered together, with a stress on liver func-

TABLE 2

NUMBER OF ACID-FAST BACILLI IN SPUTUM IN VARIOUS TYPES OF PULMONARY LESIONS

Type of Pulmonary Lesion	Sputum Examination for Acid-Fast Bacilli					
	Strongly Positive	About 20 Bacilli per Field	About 1 Bacillus per Field	Positive for Acid-Fast Bacilli by Concentration	Negative for Acid-Fast Bacilli	Total in Each Category
Far advanced	18	15	2	3	None	38
Moderately advanced	5	—	13	4	1	23
Minimal	1	—	3	12	14	30
Miscellaneous	—	—	1	3	5	9

TABLE 3

COMPARATIVE STUDY OF THE PERCENTAGE OF MORPHOLOGIC CHANGES IN THE LIVER BY DIFFERENT OBSERVERS

	Present Cases	Marvin *et al.*	Bindra Ban
Definite tubercles	15	13	9
Focal cellular collections	35	20	33
Focal necrosis	47	89	12
Fatty metamorphosis	27	20	35
Cirrhotic changes	27	18	39
Reticuloendothelial cell hyperplasia	57	—	6
Number of cases investigated	100	70	34

tion tests, it was found that there were 31 patients with derangement in more than four liver function tests. Of these only 12 had liver enlargement while 19 showed tuberculous lesions in the liver. This demonstrates that no significant correlation exists between these three tests.

Liver function tests with histopathology: Of 25 patients who showed definite or highly suggestive tuberculous lesions in the liver, 19 had marked derangement in liver function. In addition, 11 patients showed moderate derangement of both liver function and morphology. On the other hand, there were 5 patients with marked histopathologic changes and minimal alteration in liver function, while the reverse condition was noted in 6 patients. In the remaining cases results were not significant regarding both liver functions and morphology, for there were minor derangements in one or both. This indicates that a significant relationship exists between deranged histopathology and liver functions.

Roentgenographic features with histopathology: Of 38 patients with advanced tuberculosis, 10 showed tuberculous lesions; 18, moderate change; and 10 patients showed only slight or no change in the morphology of the liver. While in a total of 53 patients with moderately advanced or minimal lesions only 7 had tuberculous lesions, in 20 there was moderate change, and in 26 only slight change in the morphology of the liver. This shows that advancement of pulmonary lesions is significantly correlated with the extent of hepatic parenchymal involvement.

Roentgenographic features with liver function tests: Of 38 patients with advanced tuberculosis, 15 showed marked derangement; 19, moderate; and 4, only slight derangement in liver function tests. Of the 53 patients with moderately advanced or minimal lesions, 8 showed marked derangement; 5, moderate; and, 40 only slight derangement in liver function tests. This indicates that the liver functions are a better guide to the advancement of pulmonary lesions than are morphologic changes.

Bacteriologic findings and histopathology: Of 38 patients with sputum strongly positive for tubercle bacilli, 10 had tuberculous lesions in the liver; of the remaining 52, only 5 demonstrated such changes. This shows that the number of acid-fast bacilli in the sputum has some correlation with morphologic changes.

Bacteriologic findings and liver function tests: Of 38 patients with sputum strongly positive for tubercle bacilli, 21 had marked derangement in liver functions while, of the remaining 52, only 6 had such derangement. This suggests that the greater the number of acid-fast bacilli in the sputum, the greater is the derangement in liver functions.

Abdominal signs and symptoms associated with histopathology: Sixteen of 55 patients with one or more abdominal signs or symptoms had tuberculous lesions, compared with a total of 25 of 100 patients. This indicates a little greater incidence of morphologic changes in the liver when the abdomen is also involved.

Abdominal signs and symptoms associated with liver functions: Twenty-one of 55 patients with one or more abdominal signs or symptoms had marked derangement in liver functions, as compared to a total of 31 of 100 patients. Hence, slightly greater derangement in liver functions is seen when the abdomen is involved.

Discussion

Granulomatous lesions are formed in the liver in cases of pulmonary tuberculosis following hematogenous spread by way of the hepatic artery. In addition, if the pulmonary lesion is associated with intestinal tuberculosis, the miliary spread to the liver can occur through the portal vein. The lymphatic and cholangitic spread, although possible, is quite rare. The incidence of hepatic involvement in pulmonary tuberculosis as seen at autopsy has been recorded by a number of observers: Saphir (3), 80–100 per cent; Sherlock (4), 99 per cent; Lorentz (5), 99 per cent; Levenson and Karsner (6), 80 per cent; Ullom (7) and Parini (8), 63–90 per cent; Torrey (9), 63 per cent. So high an incidence of tuberculous involvement of the liver in autopsy reports in cases of chronic pulmonary tuberculosis may be due either to thorough examination of the liver microscopically by serial section or this incidence may be terminal, occurring only when the defense mechanism of the liver abruptly fails shortly before death.

The study of the morphology after needle biopsy of the liver has been undertaken by a number of observers: Cradock and Meredith (10), Cohen and Gige (11), Marvin and associates (12), and Bindra Ban (13). However, in this field the most remarkable work is done by Haex and Van Beek (1) who succeeded in diagnosing even the early cases of pulmonary tuberculosis by liver biopsy. They studied more than one hundred serial sections of a single liver biopsy piece. Of

189 biopsies they found tubercles in the liver in 176 patients, i.e., 93 per cent of the cases, the highest incidence so far recorded. Compared to this figure, other observers record an incidence of only about 10 to 15 per cent. In the present series, too, the incidence was 15 per cent. It is probable that the high resistance of the liver to tubercle formation results from the presence of Kupffer's cells.

In the present series, 3 cases of miliary tuberculosis were studied, 2 of which showed only periportal lymphocytic infiltration without any epithelioid cells, giant cells, or caseation, while in one case only reticuloendothelial cell hyperplasia was noted. In none of the 3 patients was the liver enlarged although in 2 of them liver functions were moderately deranged. These findings cast doubt on the existing view that patients with miliary tuberculosis must have definite tuberculous lesions in the liver (14).

In the present series there were 4 cases of pleurisy with effusion and one case of parenchymal infiltration with effusion. All of these patients had sputum negative for tubercle bacilli even after concentration method, but the effusion was proved to be tuberculous either by culture positive for acid-fast bacilli or by a positive node biopsy. Surprisingly enough, all of these 5 patients had tubercles in the liver. This is a very interesting finding which, according to the present study, gives great importance to liver biopsy in cases of clinically suspected tuberculous effusions. Even today, most of the cases of pleural effusion are said to be tuberculous in origin only from a clinical background supplemented by therapeutic trials. It is the intention of the present writers to continue their studies.

In the present series there were 27 patients with cirrhotic changes in the liver, either moderate or quite marked. The correlation between pulmonary tuberculosis as it affects the liver and the production of cirrhosis is a most controversial point. Some people completely deny the existence of hepatic cirrhosis in pulmonary tuberculosis, while others say that tuberculosis is a frequent and definite etiologic factor in the production of cirrhosis. Levenson and Karsner (6), Spring (15), Lorentz (5), and Saphir (3) favor the latter view, while Fraenkel, Isaac, Epplen, Kirch, Kern, Gold, and Huebschmann favor the former view (quoted from Lichtman (16)).

A few isolated facts may be pertinent here: Tuberculosis is a common complication of cirrhosis, for about 10 per cent of patients with cirrhosis die of tuberculosis (16). Some patients with pulmonary tuberculosis (chronic) develop the clinical features of portal cirrhosis without tuberculous peritonitis. Fatty infiltration of the liver is quite a common accompaniment of both tuberculosis and portal cirrhosis. The tubercles in the liver are associated with fibroblastic proliferation at their periphery and, when they heal, they lead to still greater fibrosis. As the tubercles occur in both portal spaces and the sinusoids, the fibrosis can start from both periportal and intralobular sites. Other than definite tubercles, all grades of cellular infiltration are present in the liver in tuberculosis as well as in cirrhosis. Focal necrosis is a common feature in tuberculosis. If it is fairly advanced or occurs repeatedly, then it may lead to cirrhotic changes. Malnutrition is common to both tuberculosis and cirrhosis, not only on an economic basis, but also due to anorexia, abdominal pain and discomfort, diarrhea, et cetera. When a number of

biopsies were performed at different sites in the same patient, they showed both tuberculous and cirrhotic lesions in different sections. In one of the cases in the present study, a first biopsy showed a classical tuberculous lesion while other biopsies showed typical cirrhotic lesions.

Keeping all of these facts in view, it becomes rather difficult to exclude the possibility that tuberculosis may lead to cirrhotic changes. The similar background of the two diseases, namely, pulmonary tuberculosis and hepatic cirrhosis, along with their simultaneous existence, cannot be considered merely a coincidence. Thus, it would not be entirely improbable that a chronic granulomata, which is able to produce marked fibroblastic proliferation and increased reticulin fibers, would, in the long run and after repeated attacks, produce hepatic fibrosis which might become cirrhotic.

Fatty infiltration in the liver in chronic tuberculosis may be frequently observed, particularly when associated with exudative or mixed pulmonary lesions. A great number of factors are advanced to explain the fatty change in the liver in pulmonary tuberculosis. Anoxemia is often blamed for its production. This was clearly disproved by Bindra Ban (13), who compared the pulmonary tuberculous and nontuberculous lesions affecting the liver. He found that fatty infiltration was much more marked in tuberculous cases, despite the anoxemia's being more marked in nontuberculous cases. Toxemia seems to be a more potent factor, since fatty liver frequently occurs in association with infections and improves when the toxemia lessens clinically.

Prolonged malnutrition may act as a supplementary factor by reducing the efficiency of the liver to guard itself against endogenous and exogenous toxins, for many of the patients studied came from a low economic group and were quite undernourished. Recently, disordered metabolism has been claimed also to be of importance, as observed in studies of depancreatized animals, in experimental feeding with lipotropes and antilipotropes, and in cases of alcoholism, pellagra, diabetes, et cetera (17). Saphir (3) attempted to correlate fatty liver in tuberculosis with the reduced size of the islets of Langerhans found in cases with complete fatty infiltration of the liver. In patients with tuberculous enteritis, absorption of the food material may be disturbed. Moreover these patients suffer from anorexia and the ingested food is consequently restricted in quantity and quality.

In the past no importance was given to the small, diffusely scattered areas of focal necrosis, characterized by necrobiosis of hepatic cells, with mild polymorphonuclear or lymphocytic infiltration. In the present series of 100 cases, focal necrosis was moderate in 44 cases and marked in 3. Furthermore, it was observed that patients with abdominal symptoms and signs had a greater percentage of focal necrosis than patients without abdominal signs or symptoms. Hence, these lesions are due to tuberculous toxemia, probably associated with bacterial or parasitic infection of the intestines, which is too common in India (18).

Taken singly, reticuloendothelial cell hyperplasia was the most common lesion found in the present series, for 54 patients had moderate, and 3 patients had

marked, reticuloendothelial cell hyperplasia. This is a nonspecific change, although it was invariably present in the cases in the present study, for it can be found in any granulomatous condition. A high prevalence of chronic malaria in India further lessens its importance.

Summary

One hundred cases of pulmonary tuberculosis were studied clinically, roentgenographically, and bacteriologically and, in addition, in each case a set of liver function tests were performed along with biopsy studies.

Epithelioid cell tubercles with or without giant cells or caseation, or both, were found in 15 cases, one of which was a tuberculoma. The liver was enlarged in 3 of the 15 cases, and hepatic function was moderately or markedly disturbed in 8 patients. Periportal lymphocytic infiltration was noted in 2 of the 3 cases of miliary tuberculosis studied.

Fatty infiltration, which is a common feature of tuberculosis, was seen in 27 per cent of the 100 cases. It is believed to be due to the toxicity of the tuberculous infection, associated with malnutrition and metabolic disturbances.

Focal necrosis was one of the common features observed, for it was found in 47 of the cases. The necrosis is believed to be due to the toxicity of the tuberculosis in association with unidentified intestinal bacterial or protozoan infections which are quite common in India.

Liver function tests were found to be moderately or markedly deranged in 31 of the 100 cases.

Sumario

El Desarreglo Hepático en la Tuberculosis Pulmonar

Cien casos de tuberculosis pulmonar fueron estudiados clínica, radiográfica y bacteriológicamente, ejecutándose además en cada caso una serie de pruebas de la función hepática junto con estudios biópsicos.

En 15 casos, se descubrieron tubérculos de células epitelioideas con o sin gigantocitos o caseación, o ambos, siendo uno de ellos tuberculoma. En 3 de los 15 casos, había hepatomegalia y en 8 la función hepática estaba moderada o notablemente perturbada. De 3 casos de granulia estudiados, en 2 se notó infiltración linfocítica periportal.

En 27 por ciento de los 100 casos, se observó infiltración adiposa, que es una característica común de la tuberculosis. Parece que se debe a la toxicidad de la infección tuberculosa, unida a la desnutrición y a los trastornos metabólicos.

La necrosis focal fué una de las características comunes, siendo observada en 47 de los casos. Parece deberse a la toxicidad de la tuberculosis, asociada con infecciones bacterianas o protozoarias sin identificar del intestino, que son bastante comunes en la India.

Las pruebas de la función hepática revelaron desarreglo moderado o destacado en 31 de los 100 casos.

Resume

L'altération hépatique dans la tuberculose pulmonaire

Cent cas de tuberculose pulmonaire ont été étudiés cliniquement, radiologiquement et bactériologiquement; de plus, une série de tests de la fonction hépatique en même temps que des études par biopsie ont été effectuées dans chacun des cas.

Des tubercules à cellules épithélioïdes avec présence ou non de cellules géantes ou de

caséation, ou les deux, ont été découverts dans 15 cas, dans l'un d'eux il s'agissait d'un tuberculome. Parmi ces 15 cas, 3 présentaient une augmentation de volume du foie et chez 8 malades la fonction hépatique était modérément ou considérablement altérée. Dans les 3 cas de tuberculose miliaire étudiés, l'infiltration lymphocytaire périportale a été observée dans 2 cas.

Dans 100 cas, l'infiltration graisseuse, qui est une caractéristique commune en tuberculose, a été observée dans 27% des cas.

Les auteurs attribuent ceci à la toxicité de l'infection tuberculeuse associée à la malnutrition et aux troubles du métabolisme.

Le foyer nécrotique était également une des caractéristiques communes car il a été découvert dans 47 de ces cas. Les auteurs estiment que la nécrose est due à la toxicité de la tuberculose associée à des infection intestinales non identifiées, à bactéries ou à protozoaires, qui sont très fréquentes en Inde.

Les résultats des tests de la fonction hépatique ont été trouvés modérément ou considérablement modifiés dans 31 de ces 100 cas.

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RAPID TEST OF VIRULENCE OF MYCOBACTERIUM TUBERCULOSIS BY INOCULATION OF CISTERNA MAGNA OF GUINEA PIGS[1]

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INTRODUCTION

Inoculation of guinea pigs with tuberculous material has been confined mainly to the subcutaneous, intramuscular, and intraperitoneal routes. While intracranial inoculation directly into the brain substance has been reported by a number of workers, inoculation directly into the basal cistern to induce leptomeningitis has rarely been used. Martin in 1898 (1) injected cerebrospinal fluid from a fatal case of tuberculous meningitis directly into the basal cistesn of a guinea pig. Wegeforth, Ayer, and Essick in 1919 (2) explored the cisterna magna in cats, performing 1,186 punctures without loss. Although the work of these investigators was not on tuberculosis, it materially aided development of the technique. Feldman and Magath (3, 4) in 1930 described experimental tuberculous meningitis in the guinea pig. In 1937 Smithburn (5) studied biologic titration of virulence of tubercle bacilli by intracranial injection, and in 1939 (6) the restoration of virulence by serial brain-to-brain passages in animals. Steenken (7) in 1951 injected guinea pigs intracerebrally through a trephine hole with tubercle bacilli H37Rv. Stevens, Karlson, and Feldman in 1952 (8) investigated pathogenicity of the tubercle bacillus by the spinal route, injecting between the fourth and fifth lumbar vertebrae.

The need for a routine test more sensitive than those afforded by subcutaneous, intramuscular, and intraperitoneal inoculation prompted the cisterna study. Since the advent of chemotherapy, and particularly in the past two years, many strains of *Mycobacterium tuberculosis* of low virulence have been encountered. Observation time in conventional animal tests is thus necessarily extended beyond the usual eight weeks, and often additional animal tests are required with histopathologic examination of the organs before reports can be made to the physician. Establishing a test more sensitive than the existing intramuscular or intraperitoneal standard test is, therefore, of considerable importance.

One of the most useful attributes of the guinea pig is its susceptibility to tubercle bacilli. There are remarkable differences, however, in the degree to which different organs become involved, for some of them possess native tissue resistance. The kidney, thymus, tubes and uterus, skin, and skeletal system are relatively resistant, while the lungs, spleen, and liver are susceptible. The pancreas, generally considered resistant, is completely destroyed when inoculation is by way of the intraperitoneal route. The leptomeninges covering the brain substance are extremely susceptible when the tubercle bacilli are placed directly in contact with this tissue. Rich (9) states that "there is no tissue that is natively

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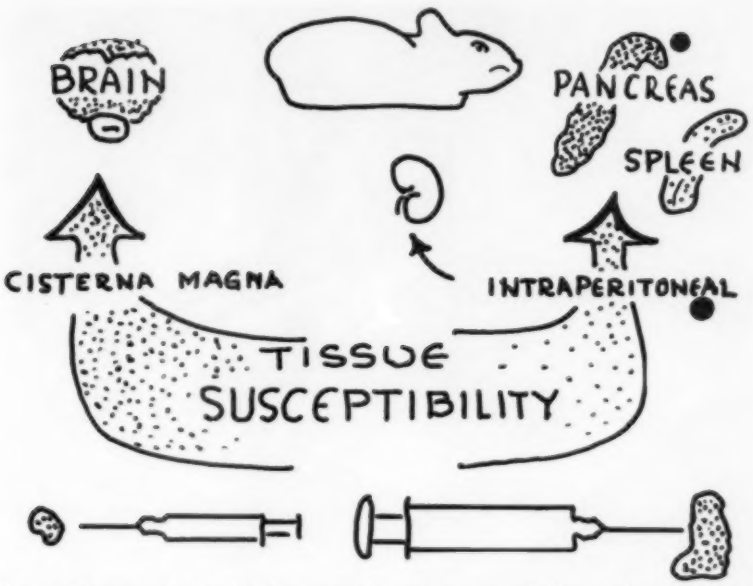

FIG. 1. Diagrammatic sketch demonstrating tissue susceptibility of the guinea pig to the tubercle bacillus.

so resistant that local progressive and destructive infection cannot be established in it if an adequate number of bacilli are deposited there." Therefore, the population of the bacilli in the dose is a factor. In figure 1 the susceptibility is shown of various guinea pig tissues to tubercle bacilli injected by different routes and in different doses. The kidney is rarely infected even by intravenous injection, while the pancreas and spleen, and the liver to a lesser extent, are involved by intraperitoneal injection. The most susceptible tissues are the leptomeninges.

MATERIALS AND METHODS

The leptomeninges of guinea pigs weighing from 180 to 200 gm. were infected with culture suspension injected into the basal cistern via the foramen magnum (figure 2). The operation could be done and even repeated numerous times on the same animal without endangering its life, as demonstrated by repeated cisternal taps on numerous guinea pigs to obtain cerebrospinal fluid for cytologic study. The deeply ether-anesthetized animal, with neck clipped and aseptically prepared, was placed in the prone position with jaws resting in a grooved head-holder designed primarily for autopsy work (10). The head was flexed by an assistant by pressing down the nose, thus thrusting the occipital portion of the head upward through the long vertical plane. Palpation revealed as landmarks the spine of the atlas and the occipital protuberance. The skin was then incised with a Bard-Parker No. 11 tapered blade in the midline dorsoposterior to the occipital protuberance below the soft spot in the depression over the ligament. The skin was pierced and incised by an upward thrust to produce a quarter-inch incision.

Inoculation was made with a three-eighth-inch, 26-gauge hypodermic needle attached

FIG. 2. Diagrammatic sketch of guinea pig brain showing landmarks for inoculation via the cisterna magna.

to a 0.25 ml. syringe. The needle was introduced through the incised skin and passed through the atlanto-occipital ligament and dura at midline in the long vertical plane at an angle to intersect a line passing through the eyes. When the resistance lessened, the needle was known to be in the cistern. A little cerebrospinal fluid could be withdrawn to further indicate that fact. One-tenth milliliter of culture suspension was then injected. The skin incision was closed with flexible collodion.

Preparation of inoculum: Cultures grown for three to four weeks on Löwenstein medium were weighed and homogenized by shaking with glass beads for fifteen minutes, then were diluted with physiologic salt solution to make 1 mg. per ml.

Animal experiment: Experiments were designed to determine relative survival time factors with a standardized dose of inoculum and the effect of a tenfold dilution on survival time and on pathologic changes in the meninges. Eleven strains of *M. tuberculosis* of varying pathogenicity for guinea pigs by routine methods of inoculation were used; two additional strains thought to be of low virulence, however, failed to show pathogenicity in the animal test. While some of the animals were allowed to die to determine an end point, others were killed at early and late paralysis. It had previously been ascertained that sufficient pathologic changes of the meninges were found for diagnosis when definite paralysis was present. Nine strains of acid-fast saprophytes were tested also to determine the effect, if any, on the leptomeninges.

Removal of the brain: The head of each animal was secured to the metal head holder (10) (figure 3) by inserting Kelly-Rankin 8-inch hemostatic forceps in the mouth and clamping the cheek firmly to the metal platform. With the head temporarily held by the forceps, an incision was made with a Bard-Parker blade through the skin from the top of the nose back along the dorsal median line to about one inch beyond the nuchal crest down the back of the neck. The skin was laid back to expose the dorsal surfaces of the frontal, parietal, and occipital bones and their lateral sutures. It was clamped firmly to the top of the platform as illustrated in figure 3. The calvaria was removed by cutting and elevating the respective bones along the lateral suture lines. The dura was incised and the brain cut transversely through the temporal lobes. The brain was elevated with a brain spatula

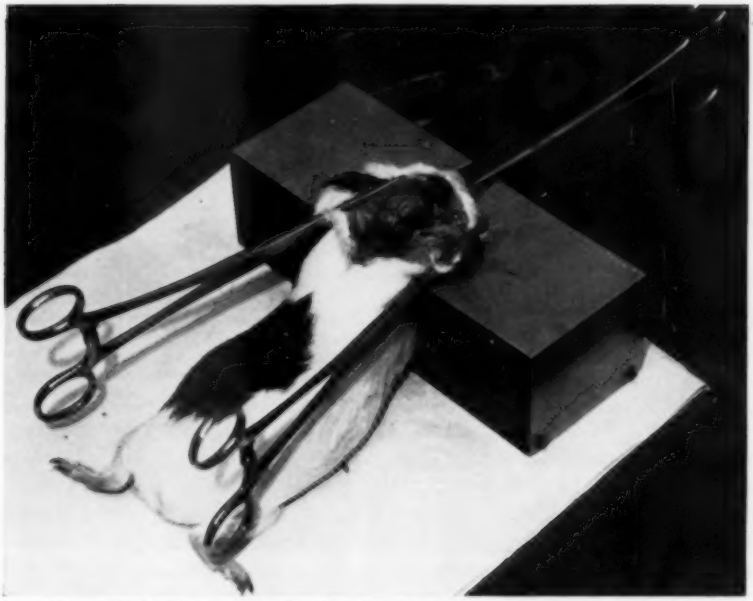

FIG. 3. Guinea pig clamped to head holder illustrating removal of brain.

the cranial nerves were cut, and finally the cord was severed from beneath and the brain was rolled out.

The brain showed grossly very little evidence of disease. There was an increase in cerebrospinal fluid pressure and the meninges over the cerebellum appeared slightly dull upon removal of the dura. If the animals were allowed to die, the brain was difficult to remove; therefore they were dispatched with chloroform when neurologic signs were severe. The cerebellum was removed in toto with a short stem of cord, and a portion of cerebrum was cut transversely through the temporal lobe since the meninges of the cerebrum were not involved. After fixation in 10-per-cent formalin, sections were obtained by dividing the brain longitudinally in the midline and cutting a slab several millimeters thick from each portion. Sections were stained with Ziehl-Neelsen stain for fifteen minutes at 60°C. for demonstration of acid-fast bacilli (11).

RESULTS

Tests with strains of M. tuberculosis of varying pathogenicity: A strain partially resistant to 0.1 γ of isoniazid per ml. of medium, when inoculated into guinea pigs cisternally in the amount of 0.1 mg. of culture suspension, produced marked paralysis in thirty-one days. Another strain morphologically typical of *M. tuberculosis* induced tuberculous meningitis in a relatively short period. One test group was paralyzed in fifteen days and was destroyed; a second group was allowed to live until severe neurologic signs were apparent. The average survival time of these two groups was fifteen and nineteen and one-half days, respectively. Histopathologic examination showed tuberculous meningitis with many acid-

TABLE 1

INTRACISTERNAL INJECTION OF THIRTY STRAINS OF M. TUBERCULOSIS IN GUINEA PIGS

M. Tuberculosis Strains			Results in Guinea Pigs	
Number Tested	Isoniazid Susceptibility	Catalase Activity	Tuberculous Meningitis	Average Survival
				days
7	Susceptible	Positive	7	25.1
12	Resistant	Positive	11	30.9
11	Resistant	Negative	7	27.3

fast bacilli in the lesions. The degree of involvement in these groups was essentially the same.

Strain H37Rv when injected in the amount of 10^{-1} mg. cisternally produced tuberculous meningitis in twenty-eight days.

Strains of lesser virulence for guinea pigs induced low-grade tuberculous lesions in routine intramuscular animal tests. Grossly the pathology was limited to the superficial inguinal and peri-iliac lymph nodes, with little involvement of the spleen. Microscopically the nodes showed inflammatory lesions typical of tuberculosis. The spleen was less involved, small lesions composed of epithelioid cells, rarely as tuberculomas with acid-fast bacilli, being present. Such animals lived well beyond the eight-week period and as a rule gave low-grade reactions when tested with 10 mg. of Old Tuberculin. These strains, inoculated via the cisterna magna into guinea pigs weighing approximately 200 gm. in doses of 0.1 ml. containing 10^{-1} mg. of culture suspension, induced tuberculous leptomeningitis which progressed to kill or completely paralyze the animal in an interval of fifteen to thirty-one days, depending upon the virulence of the strain.

Strains of low virulence usually induced a longer incubation period and paralytic phase of the disease than virulent strains, and the time lapse from early neurologic signs in the hind quarters until the animal was completely incapacitated was generally somewhat longer.

Tests with 30 strains of M. tuberculosis from patients treated with isoniazid: Guinea pigs were injected intracisternally with 10^{-1} mg. of culture suspension (table 1). Of seven catalase-positive strains susceptible to 0.1 γ of isoniazid per 1 ml. of medium, all induced tuberculous meningitis in the test animals. Twelve catalase-positive strains resistant to isoniazid induced tuberculous meningitis in 11 of 12 animals. Eleven catalase-negative strains resistant to isoniazid induced tuberculous meningitis in 7 of the 11 test animals. At the end of fifty-six days test animals still living were dispatched and examined for evidence of disease. All of these animals showed minimal leptomeningitis.

Tests with saprophytes: Nine strains of acid-fast saprophytes were tested to determine if a lesion of the leptomeninges would be produced that might be confused with that induced by virulent strains. No neurologic signs were observed and the animals inoculated with these strains continued to gain weight until they were destroyed after four months. Microscopic examination of tissue sections revealed essentially no involvement of the meninges.

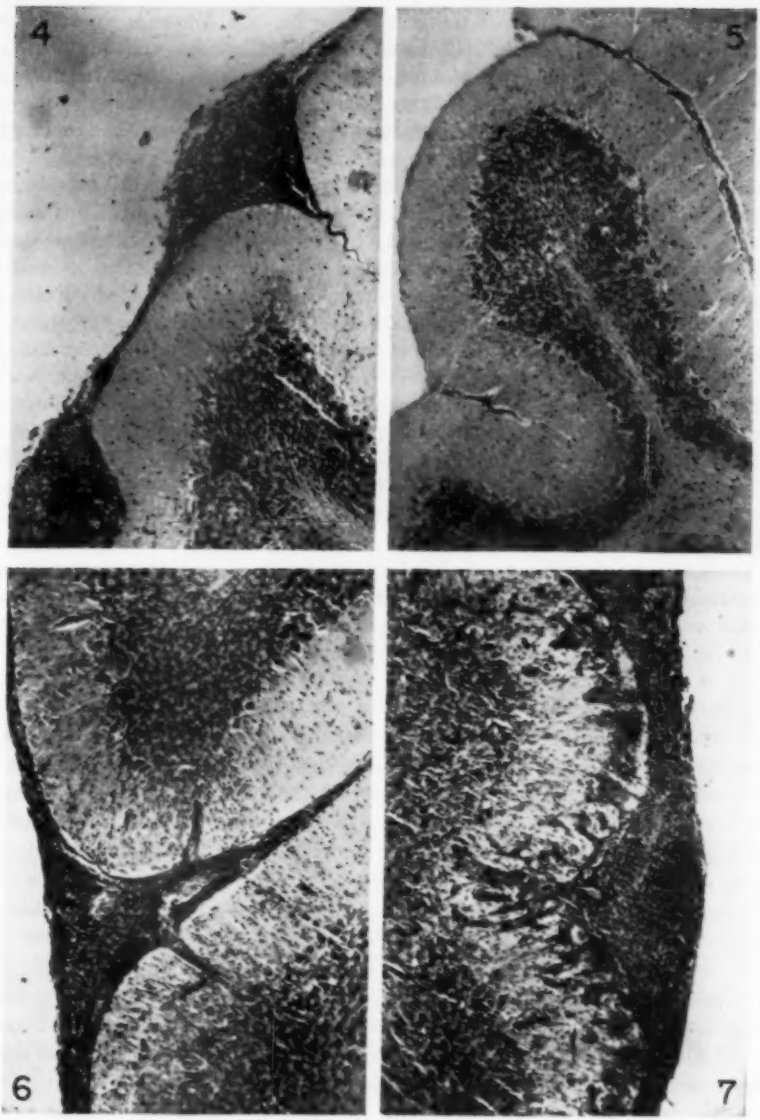

FIG. 4. Brain section from guinea pig inoculated with *Nocardia asteroides* showing granulomatous lesions.

FIG. 5. Guinea pig brain showing normal meninges.

FIG. 6. Brain section from guinea pig inoculated with *M. tuberculosis* showing tuberculous meningitis.

FIG. 7. Brain section from guinea pig inoculated with *M. tuberculosis* showing meningoencephalitis.

Tests with Nocardia: Two strains of Nocardia, one *Nocardia intracellularis* reported by Cuttino in 1949 (12), one *Nocardia asteroides*, and one acid-fast strain unidentified, isolated in the central laboratory, were injected cisternally into 15 animals. Two animals receiving 0.1 mg. of culture suspension of the unidentified strain died in sixteen and thirty-four days, respectively. The remainder of this test was terminated at approximately four months.

Histologic examination of brain sections from the 2 animals of this test showed focal lymphocytic meningitis and purulent meningitis, respectively. The findings in 5 animals were negative. Two showed a few tuberculoid lesions of the meninges and brain substance. The 8 animals injected with the Cuttino strain showed no significant lesions histologically. The strain of *Nocardia asteroides* injected in the amount of 0.1 ml. containing approximately 1 mg. (turbidity approximating that of Pyrex glass standard No. 2.5) of culture suspension killed the test animals in five days. Histologic examination revealed multiple granulomatous lesions with suppuration in the subarachnoid. Some of these lesions extended into the brain substance per se apparently by direct extension. The choroid plexus of the fourth ventricle was also involved. Gram-Weigert's stain demonstrated branching filamentous elements extending throughout the abscesses and into the lumen of blood vessels in some instances. These lesions were not identical with those of tuberculous meningitis.

In figure 4 (*Nocardia asteroides*), a focal lesion of exudative cells, chiefly granulocytes, is shown. It is not characteristic of tuberculosis; there is little tissue reaction in the leptomeninges adjacent to the abscess.

In figure 5, normal tissue of the guinea pig cerebellum may be seen. The thin layer of cells covering the surface and dipping down into the sulci constitutes the leptomeninges or the arachnoid and pia mater considered together. The inflammatory lesion develops in this vascular tissue. The lesions are shown in figures 6 and 7. Owing to the proliferation of cells of the pia and the influx of exudative cells, chiefly histiocytes and granulocytes, the meninges are no longer recognizable per se. There is a cellular response to the tubercle bacillus. Histiocytes or macrophages in the early phases of the infection engulf bacilli and continue to move about the area for some days, during which time the inflammatory reaction develops. They then undergo morphologic changes and become the epithelioid cells which are characteristic cells of a tuberculous lesion. The granulocytes die and remain there as degenerated cells; lymphocytes and circulating monocytes to a lesser extent inhabit the lesion. The virulent bacillus finds a better environment inside the histiocyte, and thus lives longer and even multiplies in it. Strains of low virulence find the environment unfavorable for growth with the end result that fewer bacilli are found in the lesions.

Tubercle bacilli proliferate, not in the parenchymatous cells, but either within the histiocytes or outside of them in the tissue spaces, in blood sinusoids in the liver, in reticuloendothelial cells, or in the alveoli in the lung. Factors responsible for differences in organ susceptibility are not altogether understood.

Summary

Intracisternal inoculation of guinea pigs with culture suspension of *M. tuberculosis* offers a rapid test for pathogenicity. Reproducible tuberculous meningitis can be demonstrated regularly with a survival time of fifteen to thirty-one days. The degree of virulence of any particular strain of *M. tuberculosis* depends upon the susceptibility of the host, the route of inoculation, and any physiologic changes of the bacterium engendered by chemotherapy. Strains having less ability to induce generalized tuberculosis by the intramuscular route will induce protracted tuberculous meningitis by the cisternal route.

Saprophytes failed to induce demonstrable meningitis and, therefore, are not confused with the pathogenic acid-fast strains. Nocardia has, in some instances, induced tuberculoid lesions; therefore, the results are considered variable.

Sumario

Prueba Rápida de la Virulencia del Mycobacterium Tuberculosis Mediante la Inoculación en la Cisterna Magna de los Cobayos

La inoculación intracisternal de los cobayos con una suspensión de cultivos de *M. tuberculosis* ofrece una prueba rápida de la patogenicidad. Cabe descubrir así con regularidad meningitis tuberculosa reproducible con un tiempo de sobrevivencia de quince a treinta y un días. El grado de virulencia de cualquier cepa dada del *M. tuberculosis* depende de la susceptibilidad del huésped, de la vía de inoculación y de toda alteración fisiológica engendrada por la quimioterapia en la bacteria. Cepas dotadas de menos capacidad para producir tuberculosis generalizada por la vía intramuscular inducirán meningitis tuberculosa prolongada por la vía cisternal.

Los saprofitos no indujeron meningitis descubrible y, por consiguiente, no se confunden con las cepas ácidorresistentes patógenas. La nocardia ha inducido, en algunos casos, lesiones tuberculoideas, por lo cual se consideran los resultados como variables.

Resume

Test rapide de la virulence du Mycobacterium tuberculosis par inoculation de la cisterna magna chez le cobaye

L'inoculation intracisternale au cobaye d'une suspension de culture de *M. tuberculosis* constitue un test rapide de la pathogénicité. Elle permet de reproduire constamment la méningite tuberculeuse avec un temps de survie de quinze à trente-et-un jours. Le degré de virulence de n'importe quelle souche particulière de *M. tuberculosis* dépend de la réceptivité de l'hôte, de la voie d'inoculation et de toute modification physiologique de la bactérie engendrée par la chimiothérapie. Les souches, dont l'aptitude à provoquer la tuberculose généralisée est moindre lorsqu'on emploie la voie intramusculaire, produiront une méningite tuberculeuse de durée prolongée lorsque la voie cisternale sera utilisée.

Les saprophytes n'ont pu provoquer la méningite clinique, ils ne peuvent donc être confondus avec les souches acido-résistantes pathogènes. Comme dans quelques cas la nocardia a provoqué des lésions tuberculoïdes, les résultats sont considérés variables.

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CULTURAL CHARACTERISTICS AND DRUG AND BACTERIOPHAGE RESISTANCE OF AVIAN TUBERCLE BACILLI[1, 2]

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INTRODUCTION

Four bacteriophages, isolated recently from enriched soils, were found to lyse each of a large series of virulent and avirulent strains of tubercle bacilli of both human and bovine origin (1). The same phage preparations, by identical methods, failed to lyse any one of a group of avian tubercle bacilli of known virulence. This refractoriness to these bacteriophages appeared to separate the organisms of avian origin into a distinct group and seemed to offer possibilities for differentiating avian tubercle bacilli from other varieties of mycobacteria. Therefore, a more intensive study of this phenomenon was undertaken in correlation with an extensive series of observations of avian and other tubercle bacilli by more widely used traditional techniques.

EXPERIMENTAL

Cultures and sources: Twenty-eight cultures in the Olive View stock culture collection were used in these experiments. Twenty-two of these strains were received labeled *M. avium* and had been initially isolated from fowl, several had been maintained in culture for long periods of time, while the majority had been isolated relatively recently. In addition, three cultures of mammalian origin not conforming to the human or bovine types were included. The well-known human virulent culture, H37Rv, and its avirulent variant, H37Ra, were added to serve as cultural and phage typing controls. All cultures were identified by code numbers only during the study, but their other designations and sources are detailed in table 1.

Media and cultivation: The organisms used in this study were cultivated and described on a variety of solid media used in the study and characterization of the 700 strains of acid-fast bacilli of all types in the Olive View culture collection. These media, previously found useful in differentiation of other strains of mycobacteria (2, 3), were:

Egg yolk-glycerol medium—Wallenstein (4)
Egg-potato-glycerol medium—Löwenstein-Jensen (5)
Blood-potato-glycerol agar—Bordet-Gengou (Difco) (6)
Oleic-acid-albumin agar—Dubos (Difco) (7)
Tween®-albumin liquid medium—Dubos (Difco) (7)

All cultures following accession were streaked on Petri dishes of egg yolk-glycerol medium and incubated until suitable isolated colonies were obtained. All incubation was at

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[1] From the Laboratory, Olive View Sanatorium, Olive View, California; and the Department of Infectious Diseases, School of Medicine, University of California, Los Angeles, California.

[2] This study was aided, in part, by grants from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association; and from the Los Angeles, Long Beach, and San Diego Tuberculosis and Health Associations.

[3] Present address: Department of Pathology, School of Medicine, University of California, Los Angeles, California.

TABLE 1

IDENTIFICATION AND SOURCES OF MYCOBACTERIA USED

Olive View Accession Number	Name	Strain	Source	Isolation
17	*M. avium*	4676	ATCC, 1950, Washington	From stork—1930, Theobald Smith
18	*M. avium*	Kirchberg	Feldman, 1950, Mayo	Spontaneous avian tuberculosis
47	*M. sp.*	Christenson	Smith, 1950, Los Angeles	Human sputum isolate
21	*M. sp.*	*M. tuberculosis* 607	ATCC, 1950, Washington	"Koch's original strain"
70	*M. tuberculosis*	H37Ra	Steenken, 1949, Trudeau	Spontaneous dissociant, 1922-1927, Petroff
71	*M. tuberculosis*	H37Rv	Steenken, 1949, Trudeau	Human sputum isolate 1905, Baldwin
60	*M. avium*	3717	Karlson, 1950, Mayo	Spontaneous avian tuberculosis
61	*M. avium*	4110	Karlson, 1950, Mayo	Spontaneous avian tuberculosis
62	*M. avium*	4133-7	Karlson, 1950, Mayo	Spontaneous avian tuberculosis
63	*M. avium*	4121	Karlson, 1950, Mayo	Spontaneous avian tuberculosis
66	*M. avium*	3965	Karlson, 1950, Mayo	Spontaneous avian tuberculosis
75	*M. avium*	Partridge	Loomis, 1926, Olive View	Spontaneous avian tuberculosis
86	*M. sp.*	4220-1	Feldman, 1951, Mayo	Bovine skin lesion
97	*M. avium*	9077	ATCC, 1953, Washington	A. H. Frank, USDA, 1941
98	*M. avium*	7992	ATCC, 1953, Washington	A. H. Frank, USDA, 1941
540	*M. sp.* (avium)?	4109	Feldman, Mayo, 1953	Human gastric lavage
541	*M. avium*	3717	Feldman, Mayo, 1953	Spontaneous avian tuberculosis
542	*M. avium*	4133-26	Feldman, Mayo, 1953	Spontaneous avian tuberculosis
543	*M. avium*	4133-84	Feldman, Mayo, 1953	Spontaneous avian tuberculosis
544	*M. avium*	4133-85	Feldman, Mayo, 1953	Spontaneous avian tuberculosis
545	*M. avium*	4017-2	Feldman, Mayo, 1953	Spontaneous avian tuberculosis
546	*M. avium*	3965	Feldman, Mayo, 1953	Spontaneous avian tuberculosis
547	*M. sp.*	3717	Feldman, Mayo, 1953	Human sputum isolate
549	*M. avium*	4133-10	Feldman, Mayo, 1953	Spontaneous avian tuberculosis
550	*M. avium*	4121	Feldman, Mayo, 1953	Spontaneous avian tuberculosis
551	*M. avium*	4110	Feldman, Mayo, 1953	Spontaneous avian tuberculosis
627	*M. avium*	Sheard—6	Steenken, 1954, Trudeau	Spontaneous avian tuberculosis
642	*M. avium*	AI	Tracey, 1954, Toronto	Connaught Med. Res. Lab., Toronto, 1928

37° C. unless otherwise noted. The cultures were photographed and a single colony was transferred to Tween-albumin liquid medium and incubated for twenty-one days. Fresh solid media in 1-ounce plastic-capped prescription bottles were then streaked with each strain and incubated. Several tubes of liquid medium were inoculated to serve as stock suspensions for animal inoculation and seeding inocula for bacteriophage susceptibility determinations.

Because of the variation in the time of appearance of growth in the cultures used, all of the media were inspected every five days and were refrigerated when growth suitable for descriptive purposes had occurred. Fluid cultures were perpetuated by transplantation every twenty-one days. All cultures were described and photographed and the observations were tabulated in detail.

Susceptibility to chemotherapeutic agents: Approximately 0.1 ml. of vigorously growing fluid cultures was also inoculated on egg-potato-glycerol medium containing chemotherapeutic agents. Each culture was inoculated on solid media containing streptomycin (10 γ per ml.) PAS (10 γ per ml.) or isoniazid (1 γ per ml.), was incubated, and the results were read at the time when the control solid media were described.

Cytochemical testing: In a previous publication (8), one of the writers described a simple method of determining, in a single preparation, the ability of tubercle bacilli to produce "serpentine cords" and to stain red when treated with neutral red. By growing microcolonies on sterile filter paper strips applied to the surface of a nutrient solid medium, then staining the strips with an acidified neutral red solution, and treating with sodium hydroxide, the neutral red reaction of the microcolonies could be observed at the same time when the presence or absence of colonial orientation was described. All of the cultures in this study series were so grown on filter paper strips on egg yolk-glycerol medium, were treated as described above, and the strips were examined with oil immersion at a magnification of 970 ×.

Bacteriophage sensitivity testing: A description of the 4 bacteriophages used in this study has been published previously (1), with a detailed discussion of their isolation, maintenance, and methods for testing the susceptibility of acid-fast bacilli to them. In the present experiments, phages were propagated on and titrated against *M. tuberculosis* var. *hominis* (H37-Rv), which was susceptible to all 4 phages. The typing dose contained 10^6 phage particles per ml.

One milliliter of a dispersed suspension of each test organism, vigorously growing in Tween-albumin liquid medium after twenty-one days' incubation, was pipetted to the surface of Bordet-Gengou agar base medium in 180 ml. screw-capped prescription bottles. Melted and cooled modified Bordet-Gengou semisolid agar was poured and mixed quickly with the inoculum and layered over the agar surface by tipping the bottle. The bottles were incubated for five days. At this time, a single standardized loopful of each of the 4 bacteriophage suspensions was spotted on quadrants of the medium and the cultures were returned to the incubator. All cultures were then observed at intervals until growth was visible.

Animal inoculation: All cultures were grown in Tween-albumin medium for twenty-one days, following two previous transfers. At this time, the supernatant medium over the centrifuged cells was removed by pipette and the bottom growth was resuspended in sterile 0.9 per cent saline solution and shaken until the suspension was smooth. Using a photometer standardized for turbidimetry, the cultures were diluted so as to contain 10 mg. of bacilli per ml. of saline solution. One milliliter of each suspension was injected intraperitoneally into three-week-old white leghorn chicks.

Three chickens died during the course of the experiment, 2 with evidence of considerable tuberculous infection. The third chicken revealed no sign of tuberculosis. The remaining chickens were sacrificed ninety days after inoculation.

Specimens of the liver and spleen from each bird were fixed in formalin for histologic section and unfixed portions were submitted to the bacteriologic laboratory for acid-fast stains and cultures of minced tissue concentrates.

Results

The 28 strains of acid-fast bacilli employed in these experiments presented eight distinct morphologic varieties with the main differentiating characteristics that are detailed in table 2. During the period in which the strains have been observed in culture (one to eight years), repeated plantings on the same media employed in the descriptive characterizations below, have failed to reveal

TABLE 2

COLONIAL AND OTHER CHARACTERISTICS OF AVIAN TUBERCLE BACILLI AND SOME OTHER ACID-FAST BACILLI

Characters:	Avian Tubercle Bacilli								Saprophytes														Human Tubercle Bacilli							
	Typical Virulent				Atypical Virulent				Rough Nonpigmented				Smooth Pigmented				Rough Pigmented				*M. Smegmatis*				Avirulent H37Ra				Virulent H37Rv			
	E	L	B	O	E	L	B	O	E	L	B	O	E	L	B	O	E	L	B	O	E	L	B	O	E	L	B	O	E	L	B	O
Shape:																																
Round	○	○	○		○	○	○	○	○	○		○	○	○	○			×	○		○	○	○	○	○	○	○	○	○	○	○	○
Irregular				○	○		○	○	×		○	○			×	○	○	○			×	×	×		○	○			○	○	○	○
Umbilicate																									×	×						
Elevation:																																
Flat	×			○			○	○	×			○			○	○								○								○
Raised	○	○	○		○	○	×		○	○	○														○	○	○	○	○	○	○	
Domed	○	○	○	×	×				○				○	○				×	○						×							
Plateau																					○	○	○									
Mammilated									×			×					○	○														
Edge:																																
Entire	○	○	○	×		○			○	○			○	○	○			×	○						○	○	○	○	○	○	○	○
Irregular											○	○			×	○	○	○			○	○	○	○								
Spreading					○		○	○	×									○	○		○	○	○	○	○	○	○	○	○	○	○	○
Fimbriate				○				○																○								
Flat				○														○	○		○	○	○						○	○	○	
Surface:																																
Smooth	○	○	○	○	×				○				○	○	○	○		×	○		○	○	○	○								
Granular	×	×				○	○	○	×	○		○					○	○					×		○	○		○	○	○	○	○
Nodular					○						○														○	○	○		○	○		
Wrinkled																					○	○										
Microscopic:																																
Serpentine																																○
Granular				○				○				○				○								○				○				
Consistency:																																
Dry				○				○															○	○	○	○	○	○	○	○	○	○
Waxy					○	○	○	○			○	○											○	○	○	○	○	○	○	○	○	○
Moist	○	○	○		○				○		○		○	○	○	○	○	○	○		○	○										
Granular						○	○					○												✕								
Pasty																					○	○	○	○								
Flaky																									×							
Crumbling																													○	○		
Shiny	○	○	○						○	○			○		○						×	×										

| | | |

evidences of any major inconsistencies or changes in appearance. Studies in this laboratory of a large number of organisms have shown that the use of standardized media and consistent methods of inoculation, incubation, and observation will allow sufficient freedom from variations in morphologic appearance to permit adequate descriptive studies.

The most common type of acid-fast bacilli observed showed the following characteristics at six weeks of incubation:

Typical Avian Tubercle Bacilli

Egg yolk-glycerol medium—pale cream, round, domed colonies with an entire edge, smooth, moist, glistening, pasty, easily miscible in aqueous solutions, diameter usually 1 mm. or less; with increasing age, occasional raised, irregular colonies with a sharp nonspreading edge and granular surface occur, seldom larger than 1 mm., moist and noncrumbling; growth abundant. On egg-potato-glycerol medium, growth was similar, but generally more moist and pasty.

Blood-potato-glycerol agar—colonies similar to above, but tan, and, with increasing age, chocolate in color; rough colonies not observed.

Oleic-acid-albumin agar—colorless, irregular, flat-spreading minute colonies with dry surface; occasionally, a small, cream, round, domed colony with an entire edge observed; at 100 × magnification with subdued transmitted light, colonies were slightly granular and nonoriented with spreading, frondlike edges.

Tween-albumin liquid medium (at twenty-one days)—white, smooth, bottom growth with no surface growth or turbidity, dispersed gradually to form a smooth suspension.

Neutral red and "cording"—neutral red positive. A majority of cultures produced no "cording" while some produced questionable orientation throughout or occasional oriented growth in a matrix of nonoriented culture. In no case was the degree of orientation that which is seen so strikingly with virulent human or bovine tubercle bacilli.

Susceptibility to chemotherapeutic agents—resistant to streptomycin, PAS, and isoniazid.

Virulence—chickens inoculated intraperitoneally with all but two of these strains (*Nos. 545, 547*) survived three months. At autopsy, all showed discrete gross or disseminated microscopic caseous or epithelioid cell tuberculous lesions.

Strains—*Strain 18* was chosen as the prototype of this group since it has exhibited the characteristics described above constantly from the time of its accession five years ago in this laboratory. Strains apparently identical with it included *60, 61, 62, 63, 66, 541, 542, 543, 544, 546, 549, 550, 551*, and *627*.

Three other strains, similar morphologically and culturally to this prototype and exhibiting the same phage and drug resistance patterns described, were repeatedly neutral red negative and showed no "cording." Two were not virulent for chickens.

Strain 540[4] had been isolated from gastric lavage specimens in a child with a pulmonary infiltrate (9). While this strain was neutral red negative, it produced typical tuberculous disease in chickens and appeared to be a true avian tubercle bacillus.

Strain 545, isolated from a tuberculous bird, was neutral red negative and produced no avian disease on repeated trials.

Strain 547 was isolated on several occasions by Feldman from a patient with roentgenographic evidence of far advanced pulmonary tuberculosis who eventually died of his disease (10). In 1947, it produced a Yersin type of infection in rabbits and chicks

[4] Obtained from Dr. William H. Feldman, Mayo Foundation, Rochester, Minnesota.

inoculated intravenously with 0.1 mg. of cells. In this laboratory, in 1954, two trials produced no evidence of tuberculous disease in chickens when the strain was administered by either the intraperitoneal or intravenous routes.

This morphologic type was considered to be characteristic of virulent avian tubercle bacilli. Eighteen of the 28 acid-fast bacilli could be placed in this type and were identical culturally, all were resistant to phage lysis and chemotherapeutic agents, and all but two produced progressive tuberculous disease in chickens. All but one of the virulent cultures were neutral red positive and "cording" was variable. These two characteristics did not appear to be an adequate index of probable pathogenicity, as they are applied to the differentiation of human virulent and avirulent tubercle bacilli, and animal inoculation alone was considered to be the only satisfactory determinant of virulence. The two strains, which were indistinguishable from this type except in their failure to produce disease in chicks on repeated trial, were described as avirulent avian tubercle bacilli.

A second type of avian tubercle bacillus, *Strain 75*, had quite different cultural characteristics:

Atypical Avian Tubercle Bacilli

Egg yolk-glycerol medium—raised colonies with irregular edges and occasional narrow flat spreading borders, nodular, waxy, dull, but moist and noncrumbling, easily miscible in aqueous solutions, producing finely granular suspensions. Occasionally, a smooth, domed, round colony was observed, identical with the prototype, *Strain 18*. All colonies were 1 mm. or less in diameter. No smooth colonies were observed on egg-potato-glycerol medium.

Blood-potato-glycerol agar—tan, round colonies, otherwise similar to growth on egg yolk media.

Oleic-acid-albumin agar—white colonies with filiform edge, dry, smooth, dull, and minute, becoming flaky and irregular on prolonged incubation.

Tween-albumin liquid medium—white, finely granular bottom growth with finely granular film on glass walls on prolonged incubation, dispersing immediately into a finely granular suspension.

Neutral red and "cording"—neutral red positive, no "cording."

Susceptibility to chemotherapeutic agents—resistant to streptomycin, PAS, and isoniazid.

Virulence—progressive tuberculosis in chickens.

This organism, although isolated thirty years ago and subcultured irregularly until 1949 when monthly transfer was begun, produced more extensive lesions in chickens than any of the other organisms tested in this study. It was easily differentiated from the bacilli of the first group by its small, rough, nodular, and irregular colonies on all solid media and by granular aqueous suspensions. It was resistant to antimicrobial drugs and to bacteriophage, and was considered to be a culturally atypical virulent avian tubercle bacillus.

Three organisms, slow-growing but culturally dissimilar to the smooth and rough virulent avian organisms, were included in the study because they had been isolated from disease processes in animals and were all resistant to phage lysis.

Rough Nonpigmented Saprophyte

Strain 642 was initially cultured from a chicken with spontaneous tuberculosis and originally conformed to the description of a typical smooth virulent avian strain (11). It had been maintained by subculture for more than thirty years. It exhibited very rough, moist, nodular colonies on all solid media; was neutral red positive; and "noncorded." The culture was susceptible to streptomycin, but resistant to PAS, isoniazid, and to phage lysis. This strain may represent a rough avirulent mutation of an originally virulent avian culture and, despite its history of isolation from a diseased bird, it appeared to be more closely related to the saprophytes in this study which were resistant to bacteriophages.

Smooth Pigmented Saprophyte

Strain 86, isolated by Feldman from a tuberculoid subcutaneous abscess in a cow, was described by him as an atypical acid-fast organism and was not necessarily considered to be of avian origin, although true avian bacilli have been isolated by Feldman under similar circumstances (12). On all solid media, colonies were minute, smooth, and invariably bright lemon-yellow. It was neutral red positive; "noncorded"; susceptible to streptomycin; and resistant to PAS, isoniazid, and to phage lysis.

Rough Pigmented Saprophyte

Strain 47 was isolated repeatedly by C. R. Smith[5] from the sputum of a patient with moderately advanced pulmonary tuberculosis. The culture produced irregular, raised, rough, noncrumbling colonies which produced yellow-gold pigmentation at room temperature. The neutral red test was positive and there was a slight degree of orientation but no true "cording." The strain was resistant to streptomycin, PAS, and isoniazid. Although clearly atypical on close observation, its importance lay in its superficial resemblance to human tubercle bacilli and in the fact that pigment did not invariably develop.

The morphologic characteristics of these three organisms, with or without the production of pigment, combined with their inability to produce disease in chickens, appeared to separate them, as a group, from virulent and avirulent avian bacilli and they were considered to be phage-resistant saprophytes.[6]

A group of bacilli which had been received under the name of *M. avium* (13) were found to be rapid-growing saprophytes.

M. smegmatis

Strains 17, *97*, and *98* produced cream-yellow to tan colonies on solid media in five to seven days which were plateau in form with umbilicate or umbonate centers, spreading, wrinkled, with a smooth or pasty consistency, and usually 5 to 7 mm. in diameter or much larger. Prolonged incubation either at room temperature or 37° C. produced striking accentuation of the surface markings of the colonies, and new peripheral growth was marked by the development of a double-zoned edge, producing "bulls-eye" colonies. The neutral red test was negative, and there was no "cording" or orientation. The cultures were quite susceptible to streptomycin, but resistant to PAS and isoniazid. These three cultures were susceptible to all four phages, produced no disease in animals, and were identical with the stock culture of *M. smegmatis* included in the

[5] Barlow Sanatorium, Los Angeles, California.

[6] Since the completion of this study, one of the phages used, *D29*, has become adapted to *Strain 47*, which is now regularly lysed by this bacteriophage. Phages have not been successfully adapted to lyse avian tubercle bacilli.

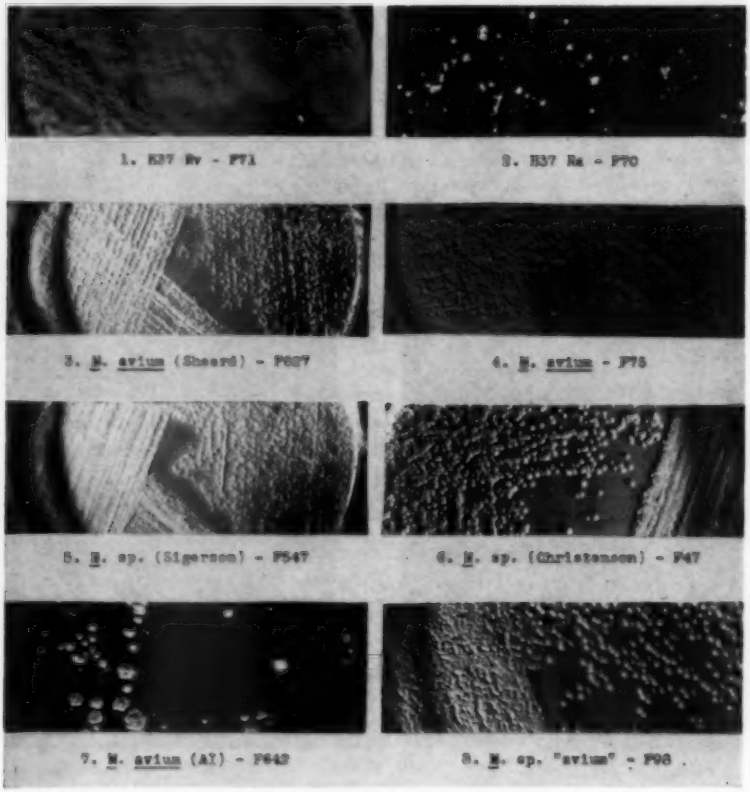
1. H37 Rv - F71
2. H37 Ra - F70
3. M. avium (Sheard) - F627
4. M. avium - F75
5. M. sp. (Sigerson) - F547
6. M. sp. (Christenson) - F47
7. M. avium (AI) - F642
8. M. sp. "avium" - F98

PLATE I

Appearance of avian and other acid-fast bacilli on egg yolk-glycerol medium.

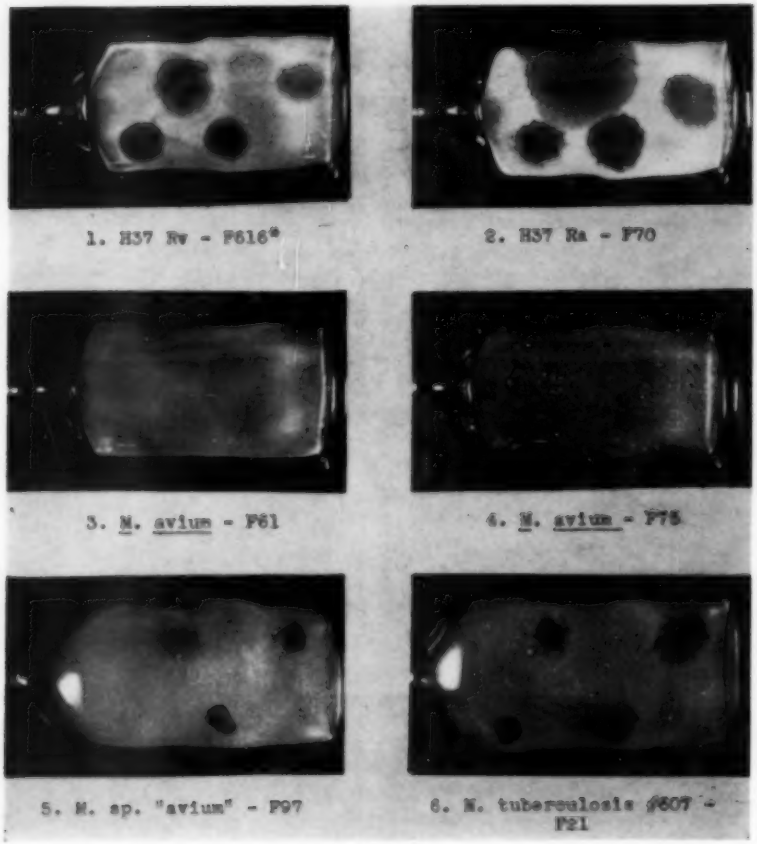

PLATE II

The appearance of phage-susceptible and phage-resistant acid-fast bacilli.

* The phage susceptibility patterns of the human tubercle bacilli (figures 1, 2) and the *M. smegmatis* types (figures 5, 6) illustrated are identical with these 4 bacteriophages. The inclusion of additional bacteriophages now in use clearly distinguishes these organisms.

series, *Strain 21*, and have all recently been so designated by Gordon (14) on the basis of much more exhaustive procedures than those used here.

Whether these rapid-growing strains of *M. smegmatis* represent faulty initial identification as *M. avium* or contamination of original true avian cultures is unimportant, but it is essential that they be labelled correctly because they conform to none of the characteristics of true avian tubercle bacilli.

Human Tubercle Bacilli

The stock cultures of human virulent and avirulent tubercle bacilli used as controls, *Strains 70* and *71*, have been described previously by many workers on a variety of substrates (15). In this study they were differentiated from the other organisms used by their slow growth, characteristic rough dry colonies, their susceptibility to all 4 of the bacteriophages and chemotherapeutic agents, and their inability to produce disease in chickens. They produced typical neutral red and "cording" reactions.

Discussion

In papers published in 1890 and 1892, Maffucci (16) demonstrated the essential dissimilarity between the acid-fast bacilli which produced disease in man and in fowl. In 1911, Griffith and his colleagues (17) introduced, in a report of the Royal Commission on Tuberculosis, an excellent summary of the cultural and pathogenic relationships and differences between mammalian and avian tubercle bacilli. Petroff and his co-workers (18), in a series of papers, showed that the avian tubercle bacillus dissociated to produce several cultural forms, each fundamentally different in virulence and producing variations in clinical and pathologic appearances. The four types of bacilli they described were:

1. Smooth (*S*) colonies, small, moist, hemispheric, readily suspended, killing chickens without gross morphologic changes except for hepatosplenomegaly, but with myriads of microscopic proliferative tubercles.
2. Flat smooth (*FS*) colonies, larger than smooth (*S*) type, wrinkled, moist and spreading, suspended with difficulty, with marked virulence, but with a more chronic clinical course, and distinct gross and miroscopic lesions.

The typical avian strains studied here appeared to belong to the Petroff *S* type colonially, but their virulence was more closely allied to the *FS* type.

3. Rough (*R*) colonies, large, dry, spreading and wrinkled, impossible to suspend, and of low virulence, with gross lesions scanty or absent.

Strain 642 appeared to conform most closely to the Petroff *R* type of organism, illustrating Saenz' observation that constant subculture may result in decrease of virulence and eventual complete loss. The ability of *Strain 75* to produce disease on repeated occasions, despite its long subculture and its tendency to roughness in growth, shows that neither Petroff's association of morphology and virulence nor Saenz' loss of virulence on subculture are always true.

4. Chromogenic (*Ch*) colonies, which were *S* type in form, but orange-yellow in color, and showed no virulence.

Saenz and Costil (19), Alexander (20), Birkhaug (21), and others further investigated the stability and pathogenicity of these specific variations. They have shown that spontaneous dissociation may occur in the host tissues, that the *R* variant may be produced after long subculture of smooth strains, and that cultures may eventually become avirulent.

Feldman (22), in 1938, ably summarized knowledge of the avian tubercle bacillus and avian tuberculosis, including a review of the cases of tuberculosis in man ascribed to *M. avium*. The atypical organisms recovered repeatedly from human subjects with pulmonary disease, which occasionally resemble avian organisms, makes some redefinition and study of the species necessary. In addition, there are a large number of strains in culture collections of acid-fast bacilli designated "avian" which are apparently not capable of producing disease in chickens. Since our knowledge of the large group of chromogenic and nonchromogenic isolates from human materials is still slight, it is unwise at present to use the term "atypical avian organism" for bacilli avirulent to chickens and other fowl, unless the bacilli resemble virulent avian tubercle bacilli in most characteristics or have been definitely derived from typical forms.

The virulence of strains of avian tubercle bacilli may be demonstrated only by suitable animal inoculation. The neutral red and "cording" determinations appeared to have little value in the assessment of avian strain virulence because of the variability of results obtained. Loss of the ability to stain with neutral red is not necessarily associated with loss of virulence for chickens (*Strain 540*) and neutral red positive organisms may be avirulent (*Strains 642, 86* and *47*).

Considerable aid in the recognition of true avian tubercle bacilli, however, may be gained by the use of phage typing as a screening procedure. If an acid-fast bacillus is resistant to phage, resembles the descriptions of avian strains in culture, and is relatively resistant to chemotherapeutic agents, it may be either a true avian tubercle bacillus or may belong to the group of apparently related organisms whose true taxonomic position is not yet clearly defined. Any acid-fast organism which is susceptible to lysis by one or more of the bacteriophages now available, however, is probably not an avian tubercle bacillus.[7]

Summary

Preliminary studies of a large collection of acid-fast bacilli indicated that true avian tubercle bacilli were not susceptible to lysis by 4 recently isolated bacteriophages active against virulent human *M. tuberculosis*. In order to assess the possibility of using this phenomenon in identification of mycobacteria, 28

[7] A phage-susceptible organism, *M. battaglini*, has been described by Penso as an atypical avian strain and was stated to produce tuberculous lesions in rabbits (23, 24). A recently acquired culture of this moderately slow-growing strain, producing smooth, round, glistening orange colonies, susceptible to the "battaglini" phage which does not lyse any of the avian tubercle bacilli described in this paper, produced miliary epithelioid tubercles in chicken and rabbit spleens on repeated trials, with recovery of the organism in the absence of typical avian tubercle bacilli. This organism is being investigated further to determine its relationship not only to virulent avian bacilli, but to the numerous smooth orange acid-fast bacilli isolated by many workers from human materials (25). *M. minetti*, although considered by Minett to be an atypical avian organism, is thought by Penso (26) to be a new species, producing tuberculous lesions in rabbits, guinea pigs, and on chorioallantoic membranes. *M. minetti* has recently been identified as *M. fortuitum* by Gordon (27, 28), including 6 strains of *M. minetti* from the writers' laboratory. These rapid-growing cultures do not resemble true avian tubercle bacilli culturally, are susceptible to bacteriophages, and produce no disease in birds, and are apparently not related to avian tubercle bacilli.

strains of acid-fast organisms, primarily avian in origin, were studied by a variety of methods including cultural morphology on solid and liquid media, neutral red and "cording" reactions, susceptibility to chemotherapeutic agents, susceptibility to phage lysis, and ability to produce disease in chickens.

Eight morphologic varieties were observed and their distinguishing characteristics described and tabulated. Eighteen strains, labeled *M. avium*, proved similar culturally, conforming to classic descriptions of avian tubercle bacilli, and all were phage resistant. Sixteen of these strains produced progressive tuberculosis in chickens, and these cultures were designated as virulent avian tubercle bacilli. The 2 remaining strains, similar in other aspects, produced no avian tuberculosis on repeated trial and were designated as avirulent avian tubercle bacilli. A second cultural type, bacteriophage and drug resistant, produced rough colonial growth and extensive tuberculosis in chickens, and was considered to be a culturally atypical virulent avian tubercle bacillus.

Three further cultural types were comprised of single strains, each from avian and mammalian sources which were phage resistant, but produced no disease in chickens and were dissimilar from the avian types culturally. These organisms, as a group, were considered to be saprophytic mycobacteria. Three organisms, identical in all respects with *M. smegmatis*, including susceptibility to all of the phages used and lack of pathogenicity for chickens, were designated as *M. smegmatis*, although they had been labeled *M. avium* on receipt. Two remaining cultural types, the control strains of *M. tuberculosis* var. *hominis*, H37Ra and H37Rv, conformed to classic descriptions and were susceptible to all of the phages used in this study.

The addition of phage typing to more traditionally used methods of study proved of value in the screening of possible avian tubercle bacilli, effecting economy in the use of animal inoculation. It demonstrated, in addition, the existence of probable avirulent avian organisms, typical in appearance but incapable of producing disease in birds. It was concluded that (*a*) if an acid-fast bacillus was resistant to any of the 4 bacteriophages used it was probably not an avian bacillus and (*b*) if an acid-fast bacillus was resistant to all 4 phages, was relatively resistant to streptomycin, PAS, and isoniazid, and resembled avian cultures colonially, the organism was either *M. avium* or was related to it.

Sumario

Características Culturales y Resistencia a las Drogas y al Bacteriófago de los Bacilos Tuberculosos Aviarios

Estudios preliminares de una gran colección de bacilos ácidorresistentes indicaron que los bacilos tuberculosos aviarios no eran susceptibles de lisis por cuatro bacteriófagos recién aislados que eran activos contra *M. tuberculosis* humanos virulentos. A fin de justipreciar el posible uso de este fenómeno para la diferenciación de las micobacterias, veintiocho cepas de microbios ácidorresistentes, primariamente de procedencia aviaria, fueron estudiadas con varios métodos, comprendiendo morfología cultural en medios sólidos y líquidos, reacciones al rojo neutro y de acordonamiento, suceptibilidad a agentes quimioterapéuticos, susceptibilidad a la lisis por bacteriófagos y capacidad para producir enfermedad en los pollos.

Observáronse ocho variedades morfológicas, cuyas características distintivas se describen y tabulan. Dieciocho cepas, rotuladas *M. avium*, resultaron semejantes culturalmente y se conformaron a las clásicas descripciones de los bacilos tuberculosos aviarios, siendo todas resistentes al bacteriófago. Dieciséis de estas cepas produjeron tuberculosis evolutiva en los pollos, designándose estos cultivos como bacilos tuberculosos aviarios virulentos. Las dos cepas restantes, similares en otros sentidos, no produjeron tuberculosis aviaria tras pruebas repetidas y fueron designadas como bacilos tuberculosos aviarios avirulentos. Un segundo tipo cultural, resistente al bacteriófago y a las drogas, produjo colonias ásperas y extensa tuberculosis en los pollos, siendo considerado como bacilo tuberculoso aviario virulento atípico culturalmente.

Tres otros tipos constaban de cepas únicas, de procedencia aviaria y mamífera, que eran bacteriófagorresistentes, pero no resultaron patógenos para los pollos, eran desemejantes culturalmente de las formas aviarias. Estos microbios, en grupo, fueron considerados como micobacterias saprofitas. Tres microbios, idénticos en todos sentidos al *M. smegmatis*, incluso en la susceptibilidad a todos los bacteriófagos usados y en la falta de patogenicidad para los pollos, fueron designados como *M. smegmatis*, aunque habían sido rotulados *M. avium* al ser recibidos. Dos restantes tipos culturales, las cepas testigos de *M. tuberculosis* var. *hominis*, H37Ra y H37Rv, se conformaron a las descripciones clásicas y fueron susceptibles a todos los bacteriófagos usados en este estudio.

La adición del tipo bacteriófágico a los métodos de estudio usados más tradicionalmente resultó de valor en el triaje de posibles bacilos tuberculosos aviarios, efectuando economías en el empleo de animales para inoculación. Demostró además la existencia de probables microbios aviarios avirulentos, de aspecto típico, pero incapaces de producir enfermedad en los pollos. Dedújose que: (*a*) si un bacilo ácidorresistente era resistente a cualquiera de los cuatro bacteriófagos usados, probablemente no era bacilo aviario y (*b*) si un bacilo ácidorresistente era resistente a todos los cuatro bacteriófagos, era relativamente resistente a la estreptomicina, al PAS y a la isoniacida y sus colonias se parecían a cultivos aviarios, el microbio era ya *Mycobacterium avium* o afín de éste.

Resume

Caractères culturaux et résistance des bacilles tuberculeux aviaires aux drogues et aux bactériophages

Des recherches préliminaires effectuées sur une grande quantité de bacilles acido-résistants ont révélé que les bacilles tuberculeux aviaires typiques n'étaient pas lysés par quatre bactériophages récemment isolés qui sont actifs contre le *M. tuberculosis* humain virulent. De façon à évaluer la possibilité d'utiliser ce phénomène dans l'identification des mycobactéries, vingt-huit souches de germes acido-résistants, principalement d'origine aviaire, furent étudiés sous divers aspects; y compris la morophologie culturale sur les milieux solides et liquides, les réactions au rouge neutre et la formation de cordes, la sensibilité à la lyse par les phages et l'aptitude à produire l'affection chez le poulet.

Huit variétés morphologiques ont été observées et leurs caractères distinctifs ont été décrits et classifiés. Dix-huit souches, désignées comme *M. avium* ont démontré leur similarité culturale, elles étaient conformes aux descriptions classiques du bacille tuberculeux aviaire et elles étaient toutes résistantes aux phages. Seize de ces souches ont déterminé une tuberculose évolutive chez le poulet, elles furent dénommées bacilles tuberculeux aviaires virulents. Les deux autres souches similaires sous d'autres aspects n'entra'naient pas la tuberculose en dépit d'essais répétés, elles furent dénommées bacilles tuberculeux aviaires avirulents. Un deuxième type cultural, résistant aux bactériophages et aux drogues produisait des colonies rugueuses et une tuberculose étendue chez le poulet, il fut considéré comme bacille tuberculeux aviaire virulent à colonies atypiques.

Trois autres types culturaux se composaient de souches différentes, chacune d'origine aviaire et mammifère et résistante aux phages, mais elles ne déterminaient pas la tuberculose chez le poulet et différaient des types aviaires dans les cultures. Ces microorganismes,

en tant que groupe, furent considérés comme des mycobactéries saprophytes. Trois micro-organismes, identiques en tout point au *M. smegmatis*, y compris la sensibilité à tous les phages utilisés et l'absence de pathogénicité pour le poulet furent dénommés *M. stegmatis*, bien qu'ils aient été désignés sous le nom de *M. avium* à l'arrivée au laboratoire. Les deux autres types culturaux, les souches-témoins *M. tuberculosis var. hominis*, H37Ra, H37Rv, étaient conformes aux descriptions classiques et sensibles à tous les phages utilisés dans cette étude.

L'identification par la méthode des phages, qui s'ajoute aux méthodes plus traditionnelles, s'est montrée de grande valeur dans la différentiation des bacilles tuberculeux pouvant avoir une origine aviaire en permettant de restreindre l'emploi de l'inoculation chez l'animal. De plus cette méthode a permis de démontrer l'existence de bacilles aviaires pouvant être avirulents dont l'aspect est conforme au type, mais qui sont inaptes à produire la maladie chez l'oiseau. Les auteurs concluent: a) si un bacille acido-résistant s'est montré résistant à l'un ou l'autre des quatre bactériophages utilisés c'est qu'il n'est probablement pas un bacille aviaire; b) si un bacille acido-résistant est résistant aux quatre phages, relativement résistant à la streptomycine, au PAS et à l'isoniazide et dont les colonies ressemblent aux cultures de bacilles aviaires, le germe est un *Mycobactérium avium* ou un bacille apparenté.

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CHARACTERIZATION OF ATYPICAL MYCOBACTERIA AND OF NOCARDIA SPECIES ISOLATED FROM CLINICAL SPECIMENS[1]

I. Characterization of Atypical Mycobacteria by Means of the Microcolonial Test

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INTRODUCTION

The significance of atypical mycobacteria, which appear to be implicated in pulmonary disease resembling tuberculosis, has been the subject of much investigation in recent years (1–10). In 1935, Pinner (11) recognized the fact that not all chromogenic mycobacteria were purely saprophytic. He observed erratic chromogenicity and variable guinea pig virulence in his cultures. He abandoned the question of the taxonomic position of these organisms, as there were no adequate *in vitro* or *in vivo* classification criteria which could be applied with assurance to this group.

In 1954, Timpe and Runyon (1, 2) reported on a systematic program for assembling a large collection of atypical mycobacteria in order to establish common and differential characteristics of these organisms and to correlate these data with the clinical origins of the bacteria in question. These workers were able to distinguish three main groups of atypical mycobacteria on the basis of mouse pathogenicity pigment production, and consistency of the colonies. They recognized two main types of chromogenicity. In one type, the colonies grown in the dark were not pigmented and developed color only after exposure to light (photochromogenicity). In the other type, pigment was produced in the dark (skotochromogenicity). Nonpigmented or very weakly pigmented atypical mycobacteria have also been observed (8, 12, 13).

In the laboratory of the present investigators, numerous strains of mycobacteria have been examined by means of the microcolonial test (14, 15) which permits determination of neutral red reaction and "cording" in one simple procedure. In the early presentations of this test no attempt was made to distinguish between the various degrees of "cording" observed. It was noted, however, that some BCG strains and isoniazid-resistant human tubercle bacilli exhibited a looser type of "cording" than is seen with typical virulent human *M. tuberculosis*. A group of chromogens was described as being neutral red positive, but not "corded." Since that time a wider variety of chromogenic mycobacteria have been examined by this technique and intermediate morphologic types observed.

It is the purpose of the present paper to characterize atypical mycobacteria according to their behavior in the microcolonial test and to correlate these results with Timpe and Runyon's observations.

[1] From the Microbiological Research Laboratory, Veterans Administration Hospital, San Fernando, California.

FIG. 1. Molecular filter membrane strip after sterilization (left) and after being placed on Löwenstein-Jensen medium. Note that the membrane takes up some of the dye in the medium.

MATERIALS AND METHODS

Bacterial Strains Employed

This study was performed on a collection of atypical mycobacteria, some of which were isolated in the clinical and research laboratories of this hospital, and the remainder of which were supplied through the kind cooperation of several investigators.[2]

For reference purposes, two stock strains of typical *M. tuberculosis* var. *hominis*, one isoniazid susceptible and the other isoniazid resistant, were included in the survey. Both had been isolated from resected lung lesions. For a more complete survey of stock classified strains of Mycobacterium species, and wild type tubercle bacilli *see* Krasnow, Wayne, and Salkin (15).

Cultivation: Millipore filter membranes were cut into strips measuring approximately 6 by 12 mm., placed in a Petri dish, and autoclaved at 10 pounds for ten minutes. After cooling, the strips were placed on the surface of Löwenstein-Jensen medium slanted in 30 ml. prescription bottles (figure 1).

Colonies from cultures of the test organisms on egg medium were emulsified in sterile distilled water or in 0.5 per cent bovine albumin fraction V by agitating with glass beads. The suspensions were diluted until barely opalescent, and additional 1:100 dilutions were made from these. A few drops of the opalescent suspensions and of the 1:100 dilutions were spotted onto membranes and permitted to flow over them and onto the adjacent medium. The bottles were capped tightly and incubated upright in the dark for twelve days or until growth was barely perceptible to the naked eye.

Staining: The membrane culture strips were stained as described previously (14). Certain mechanical improvements have been made for handling larger numbers of filter membrane strips conveniently. A Lucite® staining unit has been constructed by Mr. W. J. Juarez of this laboratory; this unit can be used to stain up to fifteen separate strips at a time. It consists of a rectangular Lucite box with three flat-bottomed troughs running

[2] Mrs. Frances Whitcomb of the Veterans Administration Hospital, Houston, Texas; Dr. Vera L. Sutter of the Wadsworth Hospital, Veterans Administration Center, Los Angeles, California; Dr. Ernest H. Runyon of the Veterans Administration Hospital, Sunmount, New York; Dr. Seymour Froman of the Olive View Sanatorium, Olive View, California; Mr. Ralph Hedberg of the Veterans Administration Hospital, Albany, New York; and Dr. Ann Pollak of the School of Medicine of the University of Kansas.

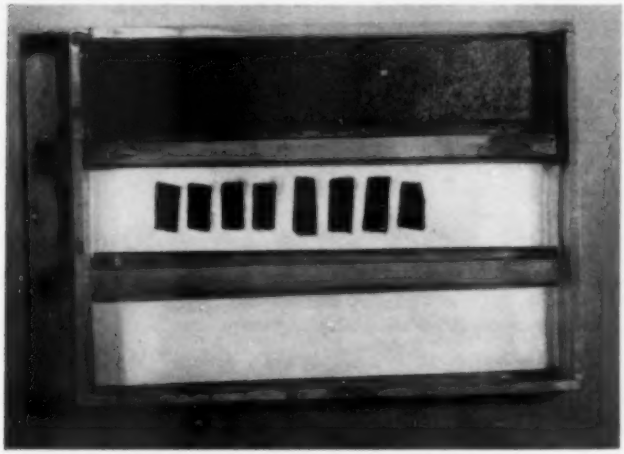

FIG. 2. Neutral red differential staining technique being performed on filter membrane cultures. The membrane strips have been exposed to the NR-6 dye (top row) and are now being decolorized on the center, alkaline paper pad. The bottom paper pad is saturated with 95 per cent ethanol. Note wells at ends of each trough for maintaining a supply of reagents to the straining pads.

its length. At both ends of each trough there is a well. Strips of Whatman 3 MM chromatography paper are laid in the troughs and bent so that both ends dip into the wells. The two wells corresponding to one trough are filled with NR-6 stain.[3] The wells corresponding to the middle trough are filled with one-tenth saturated aqueous sodium carbonate. It is the practice to maintain a saturated solution by keeping a bottle of solution over excess sodium carbonate crystals and diluting an aliquot 1:10, as needed. The wells corresponding to the last trough are filled with 95 per cent ethanol. Capillary action then draws the reagents into the entire paper pads (figure 2).

The membrane culture strips to be stained were placed, growth side up, on the paper pad saturated with NR-6 solution for ten minutes. They were then transferred to the middle, alkaline, paper pad for five minutes, and were finally transferred to the alcohol pad for *not more* than one minute. Excessive exposure to the alcohol may de-stain some neutral red positive colonies. One to three strips were placed on slides and dried at 37° C. for at least thirty minutes. In practice, these slides are placed in large test tubes for drying to prevent the membranes from being blown off accidentally.

The dried preparations were examined under reflected light with low magnification to establish the presence of colonies. This is most important when dealing with neutral red negative colonies.

After the dry preparation had been adequately studied, the membrane was mounted in Permount® under a cover slip and re-examined with low power by transmitted light. Neutral red positive colonies can be detected readily and the presence and degree of "cording" may be determined. This should be confirmed by observation at higher magnification.

Cytochemical test: The Dubos-Middlebrook neutral red cytochemical test (17) was also run on the various strains for comparative purposes.

Animal inoculation: The inability of most atypical mycobacteria to produce typical

[3] NR-6: Saturated solution of neutral red in 95 per cent ethanol, filtered, and one volume of 12 *N* hydrochloric acid added to 99 volumes of dye solution (16).

disseminating disease in guinea pigs is well established (12); therefore, only a limited number of the test strains in the present study were inoculated subcutaneously into guinea pigs.

Intradermal inoculation of mycobacteria into guinea pigs has been proposed and studied as a virulence test (18–20). A number of the strains were tested by this technique. The cultures were grown in Dubos Tween®-albumin broth and diluted to a concentration of approximately 0.1 mg. moist weight of cells per ml. The ventral surface of the test animal was shaved, and 0.1 ml. of the cell suspension was inoculated intradermally into one of the four quadrants. The animals were examined weekly for six weeks and were then sacrificed and examined at autopsy.

Many of the bacterial strains were also tested for mouse virulence. Approximately 0.5 mg. moist weight of bacilli in suspension in 0.2 ml. of Dubos Tween-albumin broth was inoculated intravenously into each of 2 mice. The mice were from four to seven weeks old and were of the local California Caviary albino strain. Animals which survived ten weeks after inoculation were sacrificed and examined at that time.

Results

The most immediate result obtained from employment of the microcolonial test with chromogens was the determination of neutral red reaction. This has been discussed previously (15), but it is necessary to point out the importance of examining the dry, stained, membrane cultures by reflected light before rendering them transparent for higher power microscopy. The neutral red negative colones were yellow to brown and tended to blend with the background in wet mount preparations. It was necessary, therefore, to examine the dry stained membranes under the dissecting microscope to confirm the presence of the neutral red negative colonies. This type of examination will also prevent a source of error which may occur in cultures of neutral red negative organisms with very heavy growth. In such cultures, the sodium carbonate may fail to diffuse to the very dense central portion of the colonies during the decolorizing process, thus leaving the centers red. While examination of these cultures before mounting in Permount makes it possible to recognize this situation immediately, the addition of the mounting fluid causes the peripheral yellow portions of such colonies to disappear, and the central red portion may suggest a false positive reaction.

In truly neutral red positive cultures, the entire colony will be red. Occasionally a neutral red negative chromogen will appear dark brown and this may be confused with a positive reaction when dry but, on rendering such a preparation transparent with Permount, these dark brown colonies are readily eliminated from the neutral red positive classification.

Among the organisms classed by Runyon as photochromogenic, a positive neutral red reaction is obtained with the microcolonial test, even though the organisms may be poorly defined by the standard cytochemical test (table 1). Certain skotochromogenic and nonpigmented atypical strains are also neutral red positive on the membranes, while others are neutral red negative. It is within the neutral red positive groups that microcolonial morphology may be observed and classified by the microcolonial test.

Group I. Consistently tight "cording": Typical *M. tuberculosis* exhibited consistently tight "cording," and the colonies were composed, primarily, of threads or ropes of growth, i.e., they were "corded." Although old colonies may have so

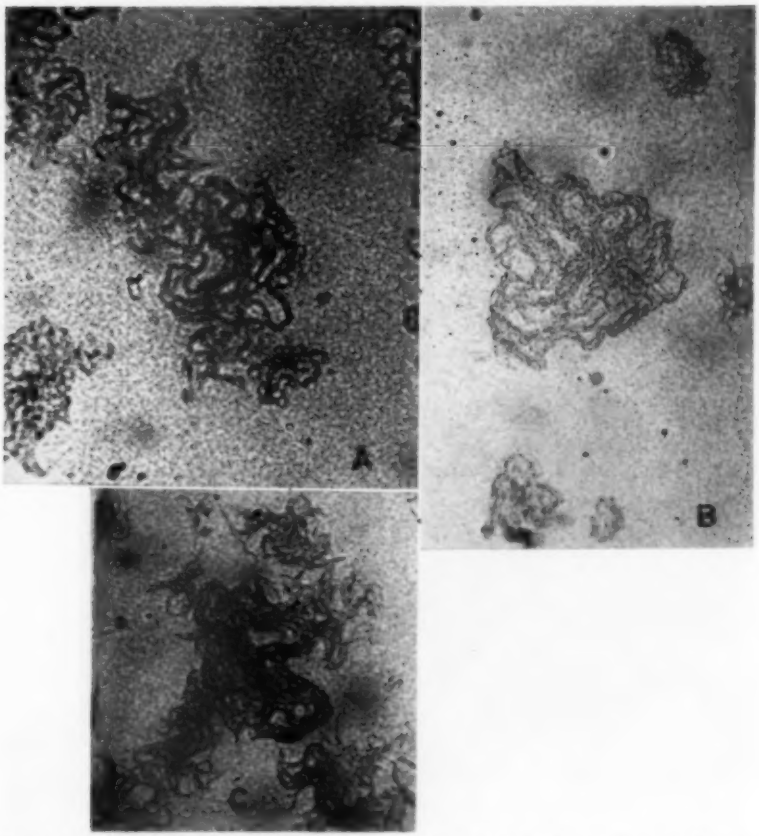

FIG. 3*A*. Photomicrograph showing tight "cords" with tendency to "wander." (× 150) *B*. Photomicrograph showing tight "cords" oriented to form a discrete colony (× 150) *C*. Photomicrograph showing broad "cords" with fewer convolutions. (× 150)

many of these threads piled on top of one another as to obscure the nature of growth, younger colonies offer no such problem. Typical virulent mammalian cultures are characterized by these thin tight threads of bacilli oriented parallel to each other; the threads may tend to "wander" away from the center of origin (figure 3*A*) or they may remain oriented about the center (figure 3*B*).

Strains which were of lesser virulence, such as isoniazid-resistant tubercle bacilli, were characterized by colonies composed of broader and less regular ropes than those described above, but the cells still exhibited parallel orientation. An individual colony would be apt to have fewer convolutions in its component "cords" than did the more virulent types of colonies (figure 3*C*).

Group II. Variable from weak "cording," but definite parallel orientation, to

tight "cording": Intensities of "cording" intermediate between the "corded" colonies characteristic of typical *M. tuberculosis* and the completely disoriented colonies which exhibit no true parallel orientation (*Group III*) were observed in colonies of certain mycobacteria. This intermediate "cording" might sometimes be seen only as a striation or "fingerprint" effect under low-power microscopic examination of transparent preparations. It was often necessary to subject such preparations to high-power examination to confirm the parallel orientation of the cells.

Here again it was possible to demonstrate gradations in intensity of orientation. Some colonies appeared to have spread rather uniformly from a central point of origin. The main colonial mass gave the impression of a thumb print implanted on it, but the periphery of the colony might be irregular and composed of broad loops (figure 4*A*) or of rather jagged projections which appeared to be

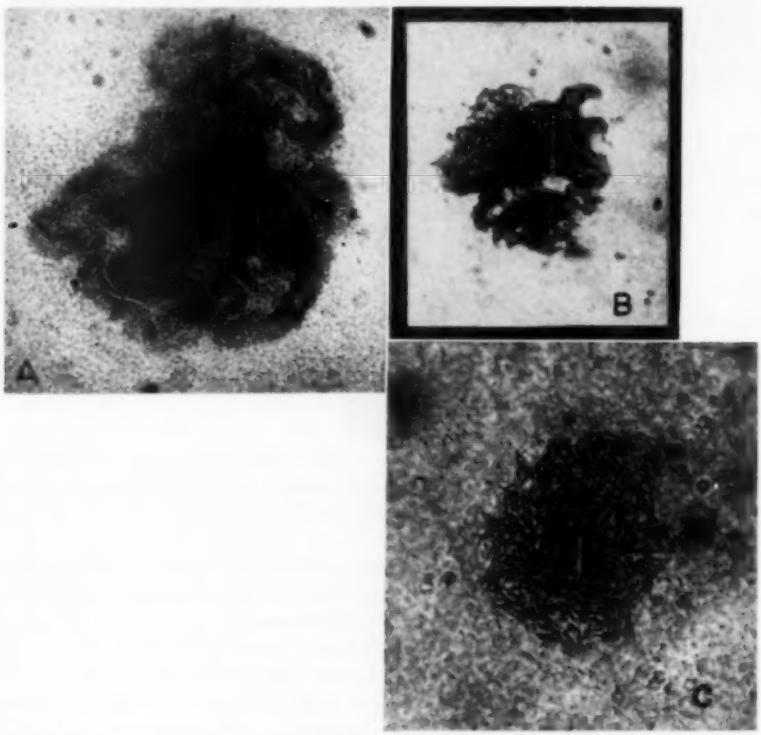

FIG. 4*A*. Photomicrograph indicating intermediate "cording" (*Group II*) with striated center and loops at periphery of colony. (× 150) *B*. Photomicrograph indicating intermediate "cording" with striated center and jagged aborted "cords" at periphery of colony. (× 150) *C*. Photomicrograph indicating intermediate "cording" at high power to confirm "fingerprint" striations. (× 600)

aborted "cords" (figure 4*B*). It was almost always necessary to examine such colonies while continuously adjusting the focus in order to confirm the striated morphology, i.e., the orientation was not all in one focal plane. For this reason, photographic illustration of this type is not very satisfactory. Again, high-power examination will confirm the parallel orientation of the component bacilli (figure 4*C*).

Group III. "Noncorded": The "noncorded" group was characterized by the absence of parallel orientation of the bacilli comprising the colony. Variations were noted within this type also.

The colonies might radiate in thick irregular branches from the center of

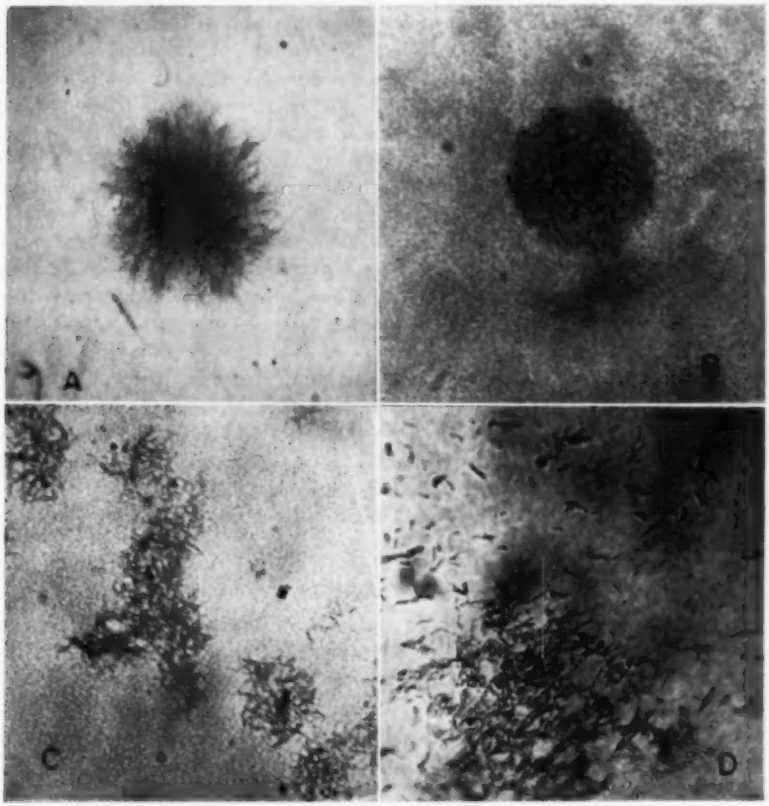

FIG. 5*A*. Photomicrograph showing "noncorded" (*Group III*) colony with thick radiating branches. (× 150) *B*. Photomicrograph showing "noncorded" colony with homogeneous texture. (× 150) *C*. Photomicrograph showing "noncorded" colony with irregular contour, suggesting a very weak "cord" formation. (× 150) *D*. Photomicrograph showing "noncorded" colony seen in figure 3*C*, examined at high power to confirm the lack of orientation of the bacilli. (× 600)

origin (figure 5*A*). This might be confused, on superficial examination, with "cording" but, upon examination with high power, it could be seen that each "branch" was composed of many bacilli completely disoriented with respect to each other, i.e., the striated organization was lacking. Some colonies might have fairly regular edges and a homogeneous center composed of cells with no apparent orientation (figure 5*B*). Other colonies seemed to be composed of a more ragged growth than was described above. The center of the colony was not homogeneous, and the growth fragments gave an impression suggestive of that seen with typical "cording" (figure 5*C*). In this instance, however, the fragments were composed of disoriented bacilli which might be seen under high power microscopy (figure 5*D*). This morphology appears to be the same as Engbaek's saprophytic type III (10). This type of growth may also appear filamentous.

Experience has shown that the intensity of "cording" or "striation" can vary with the age and moisture content of the culture and with the size of clumps present in the initial inoculum. Discrete isolated colonies are required for recognition of weak "cording." A general rule is to stain the preparations as soon as any growth is detected on the membrane. Old cultures, or those inoculated with poorly homogenized bacillary suspensions, may yield colonies which cannot be interpreted, or might be falsely interpreted (in the case of a weakly "corded" *Group II* strain) as a "noncorded" type. Whenever there is any doubt in the reading of an apparently "noncorded" strain, the test should be repeated. The reverse effect, of a *Group III* strain behaving as a false *Group I* or *II* strain, does not appear to present a problem. When clear-cut parallel orientation is observed, it may be accepted as definitive.

The neutral red reactions and morphologic group character of atypical mycobacterial strains are listed in table 1, along with their clinical source, nature of pigmentation, and reaction to the cytochemical test of Middlebrook and Dubos. A drug-susceptible strain of tubercle bacilli and one which is resistant to 100 γ of isoniazid per ml., both of which were isolated from resected pulmonary lesions, are included for reference. The groups are listed according to the three main morphologic classifications described above.

Photochromogens

Runyon states, "The similarity of the photochromogenic strains . . . lends support to the probability that we are dealing here with one pathogen" (12). The uniformity of this group is supported by the microcolonial characteristics of these organisms as listed in table 1. Although the photochromogens showed some variation in reaction to the Middlebrook-Dubos cytochemical test, they were all neutral red positive by the microcolonial technique. In addition, all exhibited some degree of parallel orientation and "cording." In some cases the parallel orientation was detected only under high magnification while, in others, frank, although somewhat loose, "cording" was seen. A given culture on a single test may show some colonies with frank "cords"; others with only parallel orientation of the cells ("finger-print" effect); and the remaining colonies, intermediate between these two extremes.

TABLE 1

SOURCE,* PIGMENTATION, AND MICROCOLONIAL PROPERTIES OF ATYPICAL MYCOBACTERIA

Strain	Origin	Specimen	Micro-colonial Test		Standard Cytochemical Reaction	Pigment	Comments
			Neutral Red Reaction	Morphologic Group			
B-2947	San Fernando	Resection	+	I	+	None	Typical *M. tuberculosis*
W-49	San Fernando	Resection	+	I	+	None	Resistant to 100 γ of isoniazid per ml.
W-47	San Fernando	Sputum	+	II	+	Photo.†	Repeated isolation
W-50	Houston	Sputum	+	II	+	Photo.	Repeated isolation
W-51	Houston	Resection	+	II	−	Photo.	
W-52	Houston	Resection	+	II	+	Photo.	
W-55P	San Fernando	Sputum	+	II	±	Photo.	Repeated isolation
W-56	San Fernando	Sputum	+	II	+	Photo.	Repeated isolation
W-57	Albany	Resection	+	II	+	Photo.	
W-96	San Fernando	Resection	+	II	+	Photo.	Typical *M. tuberculosis* isolated from same lesion
W-128	San Fernando	Resection	+	II	±	Photo.	
W-159	Kansas	?	+	II	±	Photo.	
F-654	Olive View	Sputum	+	II	+	Photo.	Isolated twice
W-55S	San Fernando	—	+	II	+	Skoto.‡	Isolated from subculture of strain W-55P
W-53	Houston	Resection	+	III	−	Skoto.	
W-54	Houston	Sputum	+	III	−	Skoto.	Repeated isolation
W-59	San Fernando	Gastric	+	III	+	Skoto.	Typical *M. tuberculosis* previously isolated from this patient
W-60	San Fernando	Gastric	+	III	±	Skoto.	Typical *M. tuberculosis* previously isolated from this patient
W-67	Sunmount	Resection	+	III	±	Skoto.	
W-69	Sunmount	Resection	+	III	−	Skoto.	
W-72A	San Fernando	Sputum	+	III	±	Skoto.	Typical *M. tuberculosis* isolated from same specimen
W-74	San Fernando	Sputum	+	III	+	Skoto.	Typical *M. tuberculosis* previously isolated from this patient
W-75	San Fernando	Resection	+	III	+	Skoto.	Typical *M. tuberculosis* isolated from same lesion
W-78	San Fernando	Sputum	+	III	±	Skoto.	
W-81B	San Fernando	Sputum	+	III	+	Skoto.	Typical *M. tuberculosis* isolated from same specimen
W-101	Wadsworth	Gastric	+	III	±	Skoto.	
W-125	San Fernando	Gastric	+	III	+	Skoto.	
W-129	San Fernando	Gastric	+	III	+	Skoto.	Typical *M. tuberculosis* previously isolated from this patient

TABLE 1—CONTINUED

Strain	Origin	Specimen	Micro-colonial Test		Standard Cytochemical Reaction	Pigment	Comments
			Neutral Red Reaction	Morphologic Group			
F-644	Olive View	Pus	+	III	−	Skoto.	From jawline abscess
F-648	Olive View	?	+	III	−	Skoto.	
F-650	Olive View	Pus	+	III	+	Skoto.	Repeated isolation from a facial lesion
F-651	Olive View	Pus	+	III	±	Skoto.	Isolated twice from a draining cervical gland
F-652	Olive View	?	+	III	−	Skoto.	
F-653	Olive View	Pus	+	III	?	Skoto.	Abscess
W-68	Sunmount	Resection	+	III	−	None	
W-120	San Fernando	Sputum	+	III	−	None	Repeated isolation from patient with chronic pneumonia
W-131	San Fernando	Sputum	+	III	±	None	Isolated twice
W-71	San Fernando	Sputum	−		−	Skoto.	Typical *M. tuberculosis* previously isolated from this patient
W-73	San Fernando	Sputum	−		−	Skoto.	
W-98	Wadsworth	Sputum	−		−	Skoto.	
W-99	Wadsworth	Gastric	−		−	Skoto.	
W-100	Wadsworth	Gastric	−		−	Skoto.	
W-102	Wadsworth	Urine	−		−	Skoto.	Isolated twice
W-103	Wadsworth	Urine	−		−	Skoto.	
W-104	Wadsworth	Urine	−		−	Skoto.	
W-106	Wadsworth	Gastric	−		−	Skoto.	
W-107	Wadsworth	Urine	−		−	Skoto.	Isolated twice
W-108	Wadsworth	Sputum	−		−	Skoto.	
W-110	Wadsworth	Urine	−		−	Skoto.	
W-111	Wadsworth	Urine	−		−	Skoto.	
W-112	Wadsworth	Urine	−		−	Skoto.	
W-113	Wadsworth	Urine	−		−	Skoto.	
W-114	Wadsworth	Urine	−		−	Skoto.	
W-115	Wadsworth	Urine	−		−	Skoto.	
W-116	Wadsworth	Urine	−		−	Skoto.	
W-117	Wadsworth	Urine	−		−	Skoto.	

* Unless otherwise specified under "Comments," typical *M. tuberculosis* had *not* been found in the patients yielding these atypical organisms.
† Photo.—photochromogens.
‡ Skoto.—akotochromogens.

One set of guinea pigs was inoculated *subcutaneously* with nine of the photochromogenic strains. All produced subcutaneous abscesses near the site of inoculation. One strain also produced a lesion in the liver, and a second strain produced multiple lesions in liver and spleen. Tuberculin sensitivity developed in all but 2 cases.

Another group of guinea pigs was inoculated *intradermally* with nine strains of photochromogens. All developed ulcers at the site of inoculation in one to three weeks. The ulcers did not regress and enlarged regional lymph nodes were found in 7 cases at autopsy six weeks after inoculation of the animals.

Nine of the eleven photochromogens were inoculated into mice. After ten weeks, eight of the cultures had produced grossly evident tubercles in spleen, liver, and/or lung. Although no gross lesions were seen in the mice inoculated with the ninth organism (*W-57*), it was possible to culture the chromogen from the spleens.

It must be mentioned that the rapidly growing saprophyte, *M. lacticola* (S. Froman, personal communication), also exhibits photochromogenic behavior. Its very rapid growth on Löwenstein-Jensen medium (one to two days) and the fact that it is neutral red negative by the microcolonial technique serve to separate it readily from the clinically significant photochromogens.

It is of interest that one strain (*W-96*) was isolated from a resected pulmonary lesion which also yielded typical colonies of tubercle bacilli on culture. In the remainder of the cases, the photochromogens appeared to be the sole microorganisms involved.

Skotochromogens

The microcolonial test afforded a clear separation between two types of skotochromogens on the basis of neutral red reaction.

Neutral Red Negative Chromogens

Neutral red negative chromogens, which vary from moist to dry and rough, are of a pale to bright yellow color. As may be seen in table 1, they were isolated predominantly from urine and gastric lavage specimens, and there is no clear evidence that any of them were clinically significant. All were neutral red negative by the cytochemical test also.

Only four of these were inoculated subcutaneously into guinea pigs and none of the animals showed any signs of disease on autopsy at fifteen weeks. All tuberculin tests in this group were negative.

Twelve of these neutral red negative organisms were inoculated intradermally into guinea pigs. Three caused some swelling and erythema which subsided after three weeks. Ulceration never occurred nor were enlarged lymph nodes seen on autopsy.

Twelve strains were inoculated intravenously into mice and none produced any gross disease, nor were the bacteria recovered from the organs of the mice.

Adequate observation of "cording" of neutral red negative bacteria is not feasible with the microcolonial test; further classification of these neutral red negative chromogens was not attempted, although the work of Gordon and associates (23, 24) would be applicable to many of these organisms. However, a degree of serpentine growth seen within the limits of the technique with some members of this group is similar to that which may be seen with *M. phlei* (15).

Neutral Red Positive Skotochromogens

With the exception of one strain, neutral red positive skotochromogens exhibited no true "cording" or parallel orientation, i.e., they fell into *Group III*. They varied in pigmentation from pale yellow to orange.

Guinea pigs were inoculated subcutaneously with eight of the "noncorded"

neutral red positive skotochromogens. Three produced abscesses, and six caused tuberculin conversion.

Eleven strains of this "noncorded" group were inoculated intradermally into guinea pigs. Six caused ulceration at the site of inoculation. Two of the ulcers persisted for the full six weeks of the experiment; the other four regressed, but enlarged lymph nodes were found in all 4 animals at autopsy. The other five strains caused varying degrees of erythema and swelling which regressed completely after three weeks in all but one case (*W-53*), in which the swelling persisted without ulcerating to the termination of the experiment. None of the 5 animals exhibited enlarged regional lymph nodes.

Thirteen of the strains were inoculated into mice. All but *W-59* and *W-60* produced lesions in spleen, liver, and/or lung. The cultures which were pathogenic for mice varied in color from very pale yellow to orange. The two which did not produce lesions in the mouse were orange and dark yellow, respectively.

The one neutral red positive skotochromogen which did produce weak "cording" and parallel orientation is listed in table 1 as strain *W-55S*. This organism was isolated as a deep salmon-colored tiny colony growing on the surface of a larger yellow photochromogenic colony of strain *W-55P*. It is pathogenic for mice and produces ulceration on intradermal inoculation into guinea pigs. Attempts to repeat isolation from the original *W-55P* mother strain have been erratic, although the *W-55S* strain was successfully purified and passed through many generations as an apparently true substrain.

Nonpigmented strains

Only slow-growing, nonpigmented, atypical clinical isolates were included in this series. All three were neutral red positive and fell into morphologic *Group III*, i.e., they showed no true "cording" or parallel orientation.

All three were injected intradermally into guinea pigs and each caused ulceration which persisted to termination of the experiment. Strain *W-120* also caused enlarged regional lymph nodes.

Only strain *W-68* was inoculated into mice and it produced spleen and lung lesions.

It must be mentioned that the nonpigmented "radish"-saprophyte (not included in table 1) is neutral red positive (15, 20), but produced no lesions after subcutaneous or intradermal inoculation in guinea pigs or on intravenous inoculation in mice.

Discussion

In attempting to evaluate the older literature on human infection with chromogenic mycobacteria, the present writers have found it impossible to correlate many reports, since there is no assurance that any two investigators are referring to the same organisms. There is no universally accepted group of criteria by means of which these mycobacteria may be identified. Indeed, more than twenty years ago Pinner (11) discussed this same difficulty.

The work of Timpe and Runyon (1–2) has supplied the first reliable criteria

which may be used to differentiate pigmented mycobacteria and which help to remove one of the deterrents to systematic study of their clinical occurrence. These criteria include determination of the ability of the bacteria to produce pigment under specified conditions and of pathogenicity for the mouse.

The classification on the basis of photochromogenic or skotochromogenic properties is practical and valid. Certain variations have been observed within any given chromogenic strain of mycobacteria in the ability to produce pigment under apparently suitable conditions. In particular, the cultures of the Timpe and Runyon classes *1* and *3* (photochromogenic), when inoculated too heavily, have frequently failed to produce pigment after exposure to light. Occasionally also, some colonies may tend to become yellow in the dark. In spite of these minor limitations, however, the classification of chromogenic mycobacteria according to the ability to produce pigment in the absence or presence of light represents the first major step in the understanding of the clinical significance of different chromogens.

It is the opinion of the present writers that the employment of the microcolonial test will serve a similar purpose, adding two more criteria for the characterization of atypical mycobacteria with a single, simple test. These two criteria, neutral red reaction and microcolonial morphology, permit resolution of mycobacteria into a number of major groups.

A positive cytochemical reaction as originally described by Dubos and Middlebrook (17) appears to depend on two phenomena. One is the adsorption of neutral red (free base) by cellular constituents, i.e., the dye is added to the cells as free base in an alkaline solution; and the other is the ability to retain the dye *in its red form* in the presence of alkali. Thus, either of two properties of a microorganism may cause it to appear neutral red negative by the cytochemical test. The dye in its free base state may not penetrate a mask of polar substances adhering to the cells; or the dye, after reaching the appropriate lipid constituent, may not be able to bind it in the red form in the presence of alkali. The initial 50 per cent methanol washes in the cytochemical test are intended to minimize the former, masking effect. In the experience of the present investigators, however, many of the atypical mycobacteria appear to be more heavily masked by polar substances than typical *M. tuberculosis*; they often appear hydrophilic (21) and may be more readily emulsified in saline (12). It is believed that the ability to retain the red color in the presence of alkali, rather than the ability of free base dye to reach the cellular lipids, forms the essence of the positive neutral red reaction.

The microcolonial test exposes the colonies to an alcoholic solution of the hydrochloride salt of neutral red. All cells are stained, regardless of whether they have the neutral red binding lipid(s) or not. The dye in this form appears able to penetrate polar masking substances readily. The subsequent application of alkali measures only the ability of the cells to resist decolorization. It may be noted in table 1 that the discrepancies between the cytochemical and microcolonial tests lie in one direction. The microcolonial test yielded positive results with several organisms which were negative or very weakly positive when ex-

amined by the cytochemical test. It is believed that the microcolonial test is more sensitive for detecting neutral red positive organisms. The discrepancies between the tests are due to what are interpreted as false negative reactions which result partially from masking effects which prevent the dye from reaching the cellular lipids in the cytochemical test.

Two other factors also contribute to the difference between the two techniques. The microcolonial test is performed on young cultures (preferably twelve days old or less). Goldman and Goldman (22) report that young cultures of *M. tuberculosis* H37Ra are neutral red positive by this method, but become negative with age. In the present laboratory similar results have been observed among atypical mycobacteria.

The total time of exposure to alkali in the staining process is also significant. The microcolonial test employs only five minutes of exposure to alkali. Some preliminary experiments in this laboratory indicate that all mycobacteria will decolorize after sufficient exposure to alkali, but that the time required varies from seconds to days depending on the strain and age of the cultures. The neutral red negative saprophytes decolorize in seconds to a very few minutes, regardless of age.

The microcolonial test presents another advantage in terms of observation of "cording." Examination of colonies on agar by low-power microscopy is quite adequate for recognition of the strong "cording" of typical *M. tuberculosis*. The photochromogens may not reveal "cording" by this method. A stained preparation can permit better visualization of the striations and parallel orientation of these organisms. By means of the microcolonial test, intact colonies are stained and may be examined at high magnifications. Disruptions which may be caused by making smears from liquid or solid cultures are avoided.

An immediate practical advantage of the microcolonial technique is the time it saves in performance. The cytochemical test requires at least a half day of centrifugations, decantations, and resuspensions. By employing the microcolonial test it is possible to run an equal number of tests in less than one hour. Microscopic examination of the mounted specimen then permits not only recognition of neutral red reaction, but detection of "cording." This eliminates the need for preparation of separate cultures for "cording" studies and saves time in the determination of the neutral red reaction.

Examination of table 1 indicates that there are several major groups of atypical mycobacteria, as determined on the basis of the nature of pigmentation, the neutral red reaction, and the microcolonial morphology.

The limited pathogenicity of *photochromogens* for mice and guinea pigs is in agreement with the fact that they are almost invariably isolated in clinical situations in which they appear to be significant. Their neutral red reaction and "cording" as determined by microcolonial test further indicate their homogeneity.

The neutral red positive *skotochromogens* and *nonpigmented* strains are not as well defined with respect to clinical significance or animal pathogenicity. On the basis of animal pathogenicity and clinical sources, it appears that the "noncorded," neutral red positive *skotochromogens* and "noncorded," neutral red positive, *nonpigmented* strains are heterogeneous and will require further criteria

for differentiation. Lesions were produced in mice by some orange and yellow strains but not by others. Although the neutral-red positive, nonpigmented clinical isolates reported in the present study all showed some pathogenicity, the existence of the nonpathogenic radish-saprophyte proves them to be as heterogeneous as the neutral red positive skotochromogens.

The neutral red negative skotochromogens probably occur only as saprophytic contaminants in clinical materials.

All of the preceding observations on "cording" are based on the behavior of the organisms in question when grown on Löwenstein-Jensen egg medium. Goldman and Goldman (22) have reported that certain mycobacterial strains which produce poor "cords" or none at all when examined by the filter membrane method on Löwenstein-Jensen medium do produce "cords" when a semisynthetic medium is substituted for the egg medium. The present writers have repeated this work and obtained similar results. Whether further classification of atypical mycobacteria can be achieved by employing a variety of media in the microcolonial test is under investigation.

Summary

The microcolonial test for neutral red staining of mycobacteria grown on molecular filter membranes has been applied to atypical mycobacteria isolated from clinical materials. The results have been correlated with pigmentation, animal pathogenicity, and clinical source of the atypical mycobacteria.

In addition to the criterion of the neutral red reaction, it has been possible to classify the organisms in question into three main microcolonial morphologic groups. Typical *M. tuberculosis* forms tight "cords," photochromogens produce intermediate "cording," and certain skotochromogens and nonpigmented atypical mycobacteria produce disoriented "noncorded" colonies.

Sumario

Caracterización de las Micobacterias Atípicas y de las Especies de Nocardias Aisladas de Ejemplares Clínicos: I. Caracterización de las Micobacterias Atípicas por Medio de la Prueba Microcolonial

La prueba microcolonial para la coloración con rojo neutro de micobacterias cultivadas en membranas-filtros moleculares ha sido aplicada a micobacterias atípicas aisladas de ejemplares clínicos. Los resultados fueron correlacionados con la pigmentación, la patogenicidad para los animales y la procedencia clínica de las micobacterias atípicas.

Además de la pauta ofrecida por la reacción del rojo neutro, fué posible clasificar los microbios estudiados en tres grandes grupos de morfología microcolonial. Los *M. tuberculosis* típicos forman acordonamiento intermedio y ciertas micobacterias escotocromógenas y no pigmentadas atípicas producen colonias desorientadas sin cordones.

Resume

Caractérisation des mycobactéries atypiques et des nocardias isolées des prélèvements cliniques: I. Caractérisation des mycobactéries atypiques au moyen du test de la microcolonie

Le test de la microcolonie pour la coloration au rouge neutre des mycobactéries cultivées sur des membranes moléculaires filtrantes a été appliqué au mycobactéries atypiques isolées de prélèvements cliniques. Il a été établi une correspondance entre les résul-

tats et la pigmentation, la pathogénicité pour l'animal et la source clinique des mycobactéries atypiques. Outre le critère de la réaction au rouge neutre, il a été possible de classifier les germes en question en trois principaux groupes morphologiques de microcolonies. Le *M. tuberculosis* typique donne des formations en cordes serrées, les photochromogènes produisent des formations de cordes intermédiaires et certains skotochromogènes ainsi que les mycobactéries atypiques non-pigmentées produisent des colonies sans cordes et non-organisées.

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CHARACTERIZATION OF ATYPICAL MYCOBACTERIA AND OF NOCARDIA SPECIES ISOLATED FROM CLINICAL SPECIMENS[1]

II. Procedure for Differentiating Between Acid-Fast Microorganisms

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INTRODUCTION

For many years it has been known that microorganisms other than *Mycobacterium tuberculosis* are acid fast. In the past, this fact has been overlooked all too frequently, particularly in those diagnostic laboratories which are so understaffed as to make it difficult to keep up with the daily work load. Some of these acid-fast microorganisms may be mistaken for *M. tuberculosis*, even occasionally on cultures. These microorganisms include other mycobacteria, both saprophytic and so-called atypical, and certain nocardia species.

Differentiation between these groups of microorganisms is most important, for it is quite probable that many patients now undergoing tuberculosis therapy are, in reality, suffering from a different infection. There is the possibility that a patient may have been subjected to hospitalization and therapy for tuberculosis when the acid-fast bacilli and the culture positive for acid-fast bacilli obtained from his sputum were actually saprophytic mycobacteria (1, 2). There is also the possibility that the cultures isolated from a patient were atypical mycobacteria or nocardia, in which instance there would be the problem not only of differentiating between the latter two and typical *M. tuberculosis*, but also of evaluating their significance in the pathogenesis of the patient's disease.

Some nocardia species apparently may produce an infection simulating tuberculosis (3–14). Webster (13) has reviewed 44 cases of pulmonary nocardiosis and added 7 more, while McQuown (10) has stated his belief that ". . . approximately 5 per cent of patients in hospitals for tuberculosis have nocardiosis." There have been reports also of cases from which atypical mycobacteria have been isolated repeatedly and in which typical *M. tuberculosis* has not been found. Furthermore, these atypical mycobacteria have been found as the sole microorganism in resected specimens (15–21). Under such circumstances they cannot be disregarded.

Several techniques are available for differentiating between these various groups. Runyon (18) and Timpe and Runyon (19, 20) recognized that some atypical mycobacteria produce pigmented colonies in the dark (skotochromogens) while others are chromogenic only after exposure to light (photochromogens). Wayne, Krasnow, and Huppert (21) have reported a further characterization of these microorganisms on the basis of their neutral red reaction and microcolonial morphology. In this report the terminology "atypical mycobacteria" is used only for those strains of mycobacteria which are neutral red positive by the technique

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described by Wayne and his associates (21, 22). The present report is an attempt to combine these properties into a routine procedure which would enable a laboratory to recognize and classify these acid-fast microorganisms with relatively little additional work. Definite advantages should result from such a scheme in terms of communication between different laboratories and evaluation of the significance of the strains encountered.

Materials and Methods

The investigations reported here were accomplished in two parts. In the first phase, known strains were studied to select those tests which were most likely to effect a differentiation between the several groups of acid-fast microorganisms. When this information had been obtained, a protocol was established. In the second phase, cultures isolated from routine clinical specimens were differentiated from each other by means of the protocol procedures.

Strains employed: In the first part of the study, known stock cultures representative of each group of the acid-fast microorganisms were used (table 1). The virulent and attenuated *M. tuberculosis* strains, atypical mycobacteria, and saprophytic mycobacteria were selected from those included in the reports by Wayne and associates (21, 22). The nocardia were strains from the stock culture collection at the San Fernando Veterans Administration Hospital and cultures were also obtained from the following sources: Dr. E. Azarowicz, University of California at Los Angeles; Dr. N. M. McClung, University of Kansas; and Dr. L. Ajello, Communicable Disease Center, Chamblee, Georgia.

TABLE 1

Stock Cultures Used to Test the Criteria for Differentiating Between M. Tuberculosis, Atypical Mycobacteria, Rapid-Growing Mycobacteria, and Nocardia Species

Cultures	Total Number of Strains	Catalase		Microcolonial Test*		Number of Strains with Indicated Sensitivities					
						Isoniazid (5 γ/ml)			PAS (10 γ/ml)		
		+	−	Neutral Red	"Cording"	R†	P†	S†	R	P	S
M. tuberculosis	3	2	1	+	Tight	1	1	1	0	2	1
BCG	1	1	0	+	Tight	0	1	0	0	0	1
Photochromogens	8	8	0	+	Intermediate	5	0	3	7	1	0
Skotochromogens	12	12	0	+	None	11	0	1	12	0	0
Nonpigmented atypical mycobacteria	3	3	0	+	None	2	0	1	2	1	0
Rapid-growing mycobacteria	3	3	0	−	‡	3	0	0	3	0	0
Nocardia sp.	11	11	0	−	‡	11	0	0	11	0	0

* Neutral red reaction and "cording," as determined by the microcolonial test described by Wayne, Krasnow, and Huppert (21).

† "R" represents resistance; "P," partial resistance, and "S," susceptibility to the drugs at the concentrations indicated. Partial resistance was recorded when the growth on the drug-containing tube was considerably less than that on the control tube.

‡ In the microcolonial test, "cording" cannot be determined accurately with neutral red negative microorganisms.

In the second part of the study, the testing of the protocol procedures under routine clinical laboratory conditions was accomplished through the cooperation of Dr. I. Krasnow and Mrs. L. Rodger, San Fernando Veterans Administration Hospital, and of Dr. S. Froman, Olive View Sanatorium.

Susceptibility to isoniazid and para-aminosalicylic acid (PAS): The effect of isoniazid and PAS on the known stock cultures was determined with the routine method used by the clinical laboratories. Various concentrations of these two agents were incorporated in Löwenstein-Jensen medium, inoculated with one drop of an opalescent homogeneous bacillary suspension, and incubated at 37° C. for six weeks or until maximal growth was obtained on the control tube.

Chromogenicity: All cultures were grown on Löwenstein-Jensen medium in the dark at 37° C. When maximal growth was attained, the cultures were exposed for twenty-four hours to strong light from a fluorescent lamp placed directly over the cultures.

Microcolonial test: The technique described by Wayne and his associates (21) was used to determine the neutral red reaction and the presence and degree of "cording."

Cellular branching: The well of a Fisher-Littman microslide was partially filled with corn meal agar[2] so that a cover glass, when placed over the well after inoculation, would contact the upper level of the agar. The agar, after hardening, was inoculated with one loop of a homogeneous bacillary suspension and was then covered. Three sides of the cover glass were sealed with a sterile mixture of petrolatum and paraffin (50/50). The entire slide culture was placed on a bent glass rod in a Petri plate containing a piece of filter paper covering the bottom. A small amount of sterile water was added, wetting the filter paper and maintaining a saturated atmosphere. The slide cultures were incubated at room temperature and were examined microscopically at twenty-four-, forty-eight-, and seventy-two-hour intervals. Occasionally, with cultures which fragmented very early, it was found necessary to examine these at six- and twelve-hour periods.

Catalase test: The catalase test was performed on cultures on Löwenstein-Jensen medium, using the technique of Middlebrook (23).

Results

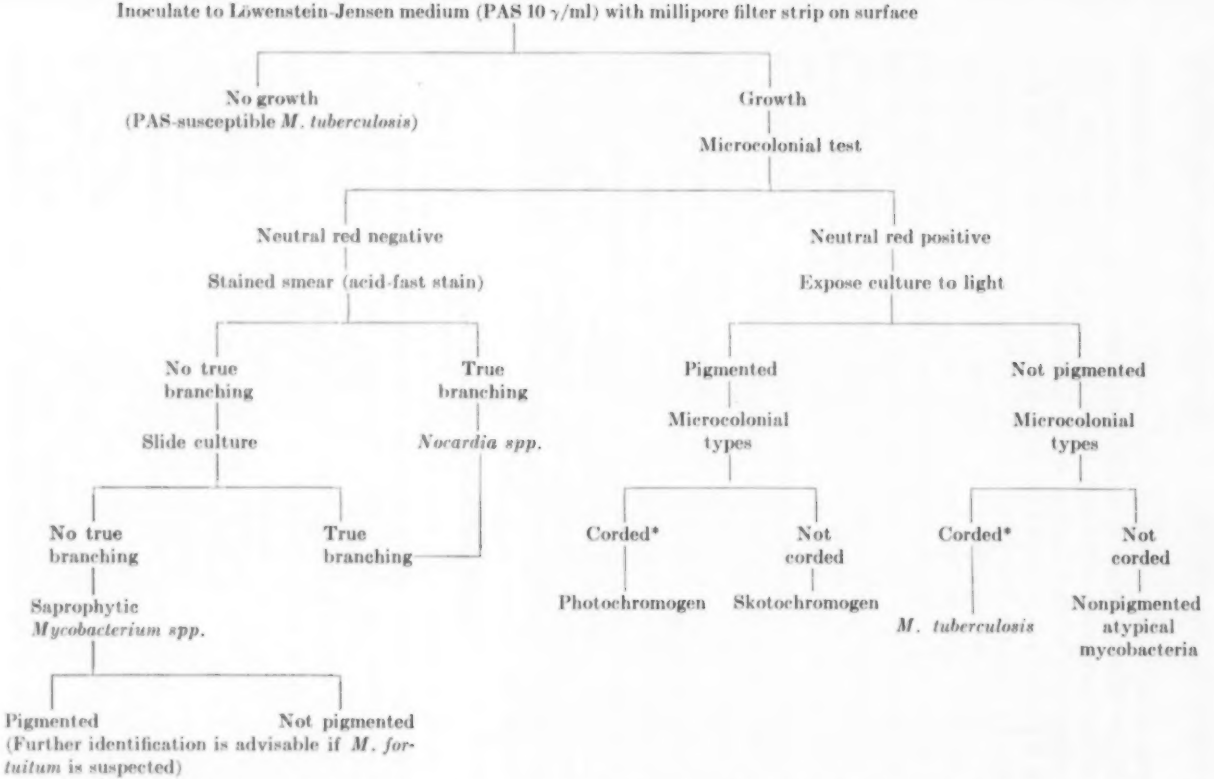
Establishing the Screening Procedures

Since the clinical laboratories tested at least one subculture from every patient for susceptibility to isoniazid and PAS, it was believed that the susceptibility to these agents would be a good starting point for establishing the screening procedures (figure 1).

The results of susceptibility tests to isoniazid were quite variable (table 1). Almost all of the atypical mycobacteria showed some resistance to isoniazid, but there was considerable overlapping at the lower drug concentrations between the less resistant atypical mycobacteria and the more resistant of the *M. tuberculosis* strains. All of the nocardia and saprophytic mycobacteria were resistant to at least 5 γ of isoniazid per ml.

With PAS the results were more consistent. All of the atypical mycobacteria, saprophytic mycobacteria, and nocardia were at least partially resistant to 10 γ of PAS per ml. Therefore, all strains resistant or partially resistant to PAS at 10 γ per ml. and all pigmented and suspicious cultures were started through the protocol. In practice, a small sterilized strip of millipore filter was placed on the surface of the medium of all 10 γ per ml. tubes of PAS used routinely in the clinical laboratory, so that the microcolonial test was begun at the same time as

[2] Obtained from Baltimore Biological Laboratories.



* The term "corded" is used here to mean all degrees of cording, including tight serpentine cords, loose cords, and cells lying parallel to each other to give the colony a "fingerprint" effect.

FIG. 1. Procedures used for recognizing different types of acid-fast microorganisms isolated from clinical specimens. (Details of the microcolonial test are described by Wayne, Krasnow, and Huppert (21).)

sensitivity testing. The technique for determining the neutral red reaction and the presence or absence of "cording" was the same as that described previously (21).

Growth on the millipore filter strip was usually perceptible in ten to twelve days. At that time the strip was removed aseptically for the microcolonial test. If the culture had grown sufficiently, it was exposed to fluorescent light overnight to determine whether pigment would be produced, if it was not present already. If growth was not sufficient, the cultures were reincubated in the dark and exposed to light at a later date. All of the nocardia and saprophytic mycobacteria were neutral red negative by this technique while all of the *M. tuberculosis* and atypical mycobacteria were neutral red positive. PAS-resistant *M. tuberculosis* cultures and nonpigmented atypical strains could be separated from the photochromogens by the failure of the first two to develop pigment when exposed to light. The atypical mycobacteria could be differentiated on the basis of the presence or absence of "cording," as determined by microscopic examination of the stained millipore filter strip. This is particularly valuable for distinguishing between *M. tuberculosis* and nonpigmented atypical mycobacteria. Details of this technique are discussed by Wayne, Krasnow, and Huppert (21).

Smears from neutral red negative cultures were stained by the acid-fast technique. With some of the nocardia strains, branching was still evident, but with many cultures no evidence of branching was found. Slide cultures were prepared from the latter group and the nocardia cultures were distinguished from the saprophytic mycobacteria on the basis of multiple branching, which occurred with the former. Some of the saprophytic mycobacteria, particularly the *M. smegmatis* type, showed branched elements, but these were short single-branched forms and not the long filaments with several branches, as may be seen in the nocardia cultures.

Application of the Screening Procedures

The results obtained with a series of 135 cultures are summarized in table 2. Of a total of 95 strains considered to be PAS-resistant *M. tuberculosis* by the clinical laboratories, one was a photochromogen, one a saprophytic mycobacterium, and three were *M. fortuitum*. In these five instances an erroneous cultural confirmation of tuberculosis would have been reported.

The remaining 40 cultures were placed in the special study group. They had not been considered as *M. tuberculosis*, but as possible atypical mycobacteria or nocardia species, before being subjected to the planned series of procedures. Eleven of these were confirmed as neutral red positive atypical mycobacteria; and these were classified further into the photochromogens, skotochromogens, and a single nonpigmented "noncorded" strain. Five of the forty were nocardia, two *N. asteroides*, and three of the *N. rubra* type. One of the *N. asteroides* cultures had been isolated from a mixed culture containing the nocardia and typical *M. tuberculosis* colonies in an equal distribution on all media. The other *N. asteroides* had been obtained repeatedly from a patient before death.

In the group of rapid-growing mycobacteria there were six strains which

TABLE 2

SUMMARY OF RESULTS OBTAINED ON CULTURES SCREENED THROUGH THE SERIES OF PROCEDURES AND ON CULTURES SENT IN FOR SPECIAL STUDY AS POSSIBLE ATYPICAL MYCOBACTERIA OR NOCARDIAS

Cultures on	Total	Pigment in		Neutral Red Reaction*		Strains Isolated						
		Dark	Light	Positive	Negative	*Mycobacterium Tuberculosis*	Photochromogens	Skotochromogens	Nonpigmented Atypical Mycobacteria	Rapid-growing Mycobacteria	*M. Fortuitum*	Nocardia Species
Procedures.....	95	0	1	91	4	90	1	0	0	1	3	0
Special study...	40	30	33	11	29	0	3	7	1	23	1	5

* Neutral red reaction as determined by the microcolonial test described by Wayne, Krasnow, and Huppert (21).

appeared to be *M. fortuitum* according to the characteristics described by Gordon and Smith (24), i.e., colonial morphology, microscopic appearance and staining reaction of the cells, growth at 37°C. but failure to grow at 45°C. or 52°C., or after four hours exposure to 60°C. When injected into mice according to the procedure described by Wells, Agius, and Smith (25), two strains failed to produce an infection, three strains each killed one of 6 mice inoculated, and the sixth strain killed 3 of 6 mice. Positive cultures were obtained from each animal which died from the infection. Only the four strains pathogenic for mice were considered to be *M. fortuitum*. The remaining two strains were classed as unidentified rapid-growing mycobacteria. All six of these strains developed some degree of branching, and originally they were placed mistakenly in the nocardia genus. It was only by careful study of the slide culture preparations that these six strains were differentiated from the nocardia.

DISCUSSION

The present studies were originated as investigations of the significance of the isolation of the atypical mycobacteria from patients with clinical diagnoses of tuberculosis. It was recognized quite early that in some cases these atypical mycobacteria were found associated with typical *M. tuberculosis*, while in others the former group were the only cultures obtained. Such findings suggested the possibility that not all of these atypical mycobacteria were equally significant. Therefore, it seemed necessary to develop techniques for recognizing and differentiating between these several types before it would be possible to evaluate their significance and relationship to tuberculosis. Any approach to this problem had to include also the isolation of these cultures from clinical material, and this made it necessary to consider all of the microorganisms which might be mistaken for either the typical tubercle bacillus or the atypical mycobacteria. The problem, therefore, was to develop a screening procedure which would identify the typical

M. tuberculosis, differentiate between the several types of atypical mycobacteria, and recognize nocardia and saprophytic mycobacteria species.

The procedure presented in this report has served this purpose well. All of these various groups of microorganisms have been encountered in routine application of a series of procedures, with relatively little additional work for the clinical laboratory. The value of such a screening procedure is apparent even in the small group of 135 cultures reported, and several interesting facts that emerged from this study may be pointed out.

The cooperating clinical laboratories had well-trained technicians and supervisors of long experience. Yet, even in these excellent laboratories, there were five instances of cultures erroneously reported as PAS-resistant *M. tuberculosis*. Unfortunately, no previous cultures from the patients involved were available, so that there was no opportunity to determine if earlier positive culture reports had been accurate. One of these five cultures was a photochromogenic atypical mycobacterium, one was *M. smegmatis*, and three were M. *fortuitum*. The isolation of the photochromogen was not unexpected; in fact, it is quite possible that all of the four strains of photochromogens, which were obtained from both phases of the study, might have been identified as typical *M. tuberculosis* except that the laboratories cooperating in this program were acutely aware of the advisability of exposing their cultures to strong light.

That four strains of rapid-growing mycobacteria should be mistaken for the typical tubercle bacillus was unexpected. Warning of this possibility has been made in the past (2). Three of these four were strains of *M. fortuitum*. One additional strain of this species was found among the cultures which had been recognized on primary isolation as not being *M. tuberculosis*. All four of these have been grouped with the "saprophytic" mycobacteria because their reaction pattern in the tests used in the protocol conforms to that of the saprophytes. It should be noted, however, that *M. fortuitum* has been isolated from lesions in man and in animals (24–26). It is possible that this microorganism may be more common than the limited number of references in the literature would indicate.

Eleven atypical mycobacteria were found in the special study group. This is not an indication of the incidence of this type, for the total number of cultures was small and was not subjected to any statistical criteria. The nonpigmented atypical mycobacterium was most unusual and probably would have been missed were it not for the microcolonial test. The isolation of this type of acid-fast bacteria illustrates the value of employing this test, not only for the reaction to the neutral red staining technique, but also for demonstrating the presence or absence of "cording."

Several additional tests could have been added to the procedures used such as animal pathogenicity, isoniazid resistance or susceptibility, and catalase activity. Each one of these tests can be helpful in the final characterization of particular strains but are of limited value as practical measures for differentiating between the four major groups of microorganisms studied, i.e., *M. tuberculosis*, atypical mycobacteria, rapid-growing mycobacteria, and nocardia.

Virulence for the guinea pig by subcutaneous inoculation would not be practicable for a number of reasons. PAS-resistant strains of *M. tuberculosis* could be

fully virulent or attenuated, if they were also isoniazid resistant. Variable pathogenicity for the guinea pig is characteristic also for strains of *N. asteroides* (27) and for the photochromogenic mycobacteria (21). The results of intradermal inoculation in guinea pigs with viable cells of the atypical mycobacteria are variable also, although most of these strains, particularly the photochromogens, do cause ulceration and enlargement of the regional lymph nodes (21). Therefore, pathogenicity for the guinea pig by subcutaneous or intradermal routes serves little useful purpose for routine screening of these microorganisms, although this property is still valuable for characterizing individual strains.

The same conclusion must be reached when considering the pathogenicity of these microorganisms for mice. The neutral red positive mycobacteria are almost uniformly virulent for this animal (21, 28), *N. asteroides* strains can cause infection which may not be lethal (29), and *M. fortuitum* appears to produce fatal disease although survival time of the mice covers a rather extended range (25).

Resistance or susceptibility to isoniazid could have been used as the starting point in the screening procedures instead of the reaction to PAS. Two major reasons led to the selection of the latter drug. In our experience resistance to PAS appeared more clear-cut than that to isoniazid. In addition, fewer strains of *M. tuberculosis* were resistant to PAS, and this reduced the number of cultures put through the screening program.

The catalase test does not distinguish between the four major groups of microorganisms with which this study was concerned. All of the strains used in Part I were catalase positive with the exception of one isoniazid-resistant strain of *M tuberculosis*. This test, however, can serve a very valuable purpose in confirming the differentiation between a nonpigmented atypical mycobacterium (neutral red positive and "noncorded") and a PAS-resistant *M. tuberculosis* (neutral red positive and weakly "corded") which is also resistant to isoniazid. The latter microorganism would be catalase negative, while all strains of the former which have been tested to date have been catalase positive.

At present it would seem that atypical mycobacteria showing some degree of "cording" are almost invariably significant while the "noncorded" strains seem to be of variable significance. Wayne, Krasnow, and Huppert (21) have reported on eleven strains of "corded" atypical mycobacteria. Ten of these were obtained either from resected specimens or as the only culture isolated, and one strain was found in a resected lesion associated with a typical *M. tuberculosis*. The same writers recorded isolations of twenty-three "noncorded" atypical mycobacteria. Sixteen of these were obtained as the sole microorganism present: four were from resected specimens; four, from abscesses; one, from the sputum of a patient with chronic pneumonia; three, from sputum; two, from gastric specimens; and two were sent in without notation of the source. The seven strains remaining of the twenty-three were all from cases in which typical tubercle bacilli had been found also.It should be noted that the degree of "cording" was determined from growth on Löwenstein-Jensen medium. Recently Goldman and Goldman (30) have shown that the degree of "cording" will vary, depending upon the medium upon which the culture has been grown.

Of the five nocardia strains isolated, two were *N. asteroides*, the species most

frequently found in cases of pulmonary nocardiosis (8, 13, 29). In one patient this actinomycete was isolated repeatedly and as the only microorganism. In the other case, the *N. asteroides* was mixed with typical *M. tuberculosis* in cultures from a lung resection. Colonies of both were found on all media and in almost equal distribution. The remaining three cultures were of the *N. rubra* type. No reports have been found in the literature in which this species has been associated with human disease, and it is believed that the three strains isolated here occurred as saprophytes in the sputum, since in two of the three instances only single colonies were found associated with a heavy growth of typical tubercle bacilli. Once again mention must be made of the fact that the two cases of *N. asteroides* isolation were not an indication of the incidence of occurrence of this microorganism. Ajello and his associates (31) have shown that this fungus does not survive acid digestion of sputum, withstands the sodium hydroxide procedure poorly, but is recovered completely after trisodium phosphate digestion. In their experiments, however, suspensions of the microorganisms were added to the sputum, and the concentration of these suspensions was adjusted so that recovery of the fungi could be expected. Consequently, considerable numbers of cells were added to the sputum, thus creating conditions which do not necessarily simulate those found in clinical material. The possibility remains, therefore, that there might have been more instances of isolation of nocardia in the studies reported in the present investigation if less drastic digestion procedures had been employed.

The results reported in this study indicate that various groups of acid-fast species can be isolated, recognized, and classified by following a screening procedure which places a relatively small additional work load on the clinical laboratory. By selecting both those cultures which have atypical colony characteristics and those which are resistant to 10 γ of PAS per ml., only a few need be screened. Laboratories which encounter this problem must be aware that not all atypical mycobacteria will grow on medium containing 10 γ of PAS per ml. The results of such tests will vary with a number of conditions, such as the medium used and the age and concentration of the cell suspension inoculated. It is necessary, therefore, in the routine examination of cultures to select for special study those cultures which are not typical of *M. tuberculosis*. When these are combined with all strains which are at least partially resistant to 10 γ of PAS per ml. most, if not all, of the atypical mycobacteria will be found. The reaction to the neutral red staining technique will separate the saprophytic mycobacteria and the nocardia species, on the one hand, from the atypical mycobacteria and PAS-resistant *M. tuberculosis*, on the other. The first two can be distinguished from each other by the presence of multiple branching in the nocardia group. The latter two can be differentiated by the production of pigment and by the presence or absence of "cording." This characterization of strains is of particular value in correlating the work of different investigators.

Summary

A number of tests have been evaluated as screening procedures, enabling a clinical laboratory to recognize several groups of acid-fast microorganisms. These groups include the typical *M. tuberculosis*, the atypical mycobacteria, the rapid-

growing mycobacteria, and certain species of nocardia. The neutral red reaction as determined in the microcolonial test will separate the first two groups (neutral red positive) from the last two (neutral red negative). The typical *M. tuberculosis* and three groups of atypical mycobacteria can be differentiated on the basis of pigment production and of presence and degree of "cording" as determined in the microcolonial test. The nocardia species can be separated from the rapid-growing mycobacteria by using the slide-culture technique; the former develop multiple branching filaments, while the latter remain unbranched or have seldom more than a few short branches.

The proposed screening procedures have been subjected to a practical test with the cooperation of two clinical laboratories. This has resulted in the ready differentiation of each of the groups of acid-fast microorganisms and, in addition, has revealed two important facts: (1) That even the best of clinical laboratories may occasionally report as *M. tuberculosis* cultures which are either rapid-growing mycobacteria or atypical mycobacteria; (2) that four strains of *M. fortuitum* and two strains of *N. asteroides* were found, some of which might otherwise have been discarded. This suggests that *M. fortuitum* may be more common than has been indicated in the literature.

Sumario

Caracterización de las Micobacterias Atípicas y de la Especie de Nocardia Aisladas de Ejemplares Clínicos: II. Procedimiento para Diferenciar los Microbios Acidorresistentes

Se han justipreciado varias pruebas como procedimientos de triaje que capacitan a un laboratorio clínico para reconocer varios grupos de microbios ácidorresistentes. Estos grupos comprenden el típico *M. tuberculosis*, las micobacterias atípicas, las micobacterias de rápido desarrollo y ciertas especies de nocardias. La reacción del rojo neutro, determinada en la prueba microcolonial, separará los primeros dos grupos (positivos al rojo neutro) de los dos últimos (negativos al rojo neutro). El *M. tuberculosis* típico y tres grupos de micobacterias atípicas pueden diferenciarse a base de la pigmentogenia y de la existencia e intensidad del acordonamiento, determinado esto en la prueba microcolonial. Las especies de nocardias pueden separarse de las micobacterias de desarrollo rápido usando la técnica de cultivo en laminillas: las primeras forman muchos filamentos ramificados, mientras que las últimas permanecen sin ramas o rara vez tienen más que algunas ramas cortas.

Los procedimientos propuestos para triaje han sido objeto de una prueba práctica con la colaboración de dos laboratorios clínicos. Esto dió por resultado la fácil diferenciación de los distintos grupos de microbios ácidorresistentes y, además, reveló dos hechos importantes: (1) hasta los mejores laboratorios clínicos pueden de cuando en cuando rotular como *M. tuberculosis* cultivos que son bien micobacterias de desarrollo rápido o micobacterias atípicas; (2) se descubrieron cuatro cepas de *M. fortuitum* y dos cepas de *N. asteroides* que, de otro modo, quizás se hubieran descartado. Esto sugiere que el *M. fortuitum* tal vez sea más frecuente que lo que indica la literatura.

Resume

Caractérisation des mycobactéries atypiques et des nocardias isolées dans des prélèvements cliniques: II. Procédés de différenciation des microorganismes acido-résistants

Quelques tests ont été évalués au titre de procédés de différenciation pour l'identification de plusieurs groupes de microorganismes acido-résistants. Ces groupes comprennent

le *M. tuberculosis* typique, les mycobactéries atypiques, les mycobactéries à croissance rapide et certaines espèces de Nocardia. La réaction au rouge neutre telle qu'elle est déterminée dans le test de la microcolonie permet de séparer les deux premiers groupes (rouge neutre positif) des deux dernières autres (rouge neutre négatif). Le *M. tuberculosis* typique et trois autres groupes de mycobactéries atypiques peuvent être différenciés sur la base de production de pigment et de la présence et du degré de la formation de cordes comme on l'observe dans le test de la microcolonie. Les nocardias peuvent être séparées des mycobactéries à croissance rapide en utilisant la technique de la culture sur lame; les premières forment des ramifications filamenteuses multiples, tandis que les dernières ne se ramifient pas ou, exceptionnellement, elles présentent des ramifications courtes et peu nombreuses.

Ces procédés de criblage ont été soumis à une épreuve pratique avec la collaboration de deux laboratoires cliniques. Ces méthodes ont permis de différencier facilement chacun des groupes de microorganismes acido-résistants et elles ont en outre révélé deux faits importants: 1) même le meilleur des laboratoires cliniques peut occasionnellement faire un rapport erroné en indiquant comme *M. tuberculosis* des colonies pouvant être des mycobactéries à croissance rapide ou des mycobactéries atypiques; 2) quatre souches de *M. fortuitum* et deux souches de *N. asteroides*, dont certaines auraient pu passer inapercues, ont été découvertes. Ceci suggère que le *M. fortuitum* pourrait être plus commun que ne l'indiquent les publications traitant de ce sujet.

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Case Reports

ACHALASIA (MEGA-ESOPHAGUS)[1]

A Condition Capable of Simulating Mediastinal Neoplasm

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INTRODUCTION

Because the mediastinum is an "anatomical crossroads," roentgenograms which demonstrate some abnormality of the mediastinal shadow present a diagnostic challenge of the highest order. Such challenges have been hurled with increasing frequency since roentgenographic examination of the chest has become a mass survey technique.

The finding of an abnormal mediastinal shadow demands urgent attention, for the "malignant potential" of this region is well recognized. To be content with anything short of a precise diagnosis is to court disaster. Therefore, it has been generally agreed that the correct approach to such lesions involves first, intensive application of all available medical diagnostic techniques; and second, prompt submission of the case to surgical exploration when other methods prove inconclusive (1–4).

In all areas of medicine in which the pendulum has swung in one direction, however, care must be taken that the caution which originally held back the pendulum is not completely discarded. The sense of urgency in dealing with mediastinal "shadows" should not lead the physician to recommendation of thoracotomy before a thorough "medical exploration" has been completed. Failure to do so may produce embarrassment at the operating table, for there are a number of nonneoplastic conditions which can masquerade as mediastinal tumors and which can be diagnosed readily through nonsurgical procedures. The entity of achalasia (mega-esophagus, cardiospasm) is one such "masquerader."

Recently, several cases have been encountered which demonstrated rather strikingly the potential of achalasia for presenting as a mediastinal tumor. Discussion of these cases will serve both to emphasize the diagnostic pitfalls which this entity can offer and to indicate the major features which enable differentiation of this condition from other mediastinal abnormalities.

CASE REPORTS

Case 1. Elt. K., a 46-year-old Negro male, was referred to the District of Columbia General Hospital because of an abnormal routine microfilm. A 14- by 17-inch chest roentgenogram was taken which was interpreted as demonstrating a mediastinal mass, and the patient was admitted to the hospital for diagnostic study.

The patient was totally asymptomatic at the time of admission. This was his first hospitalization, and he recalled no significant prior illnesses. Close questioning failed to reveal any constitutional symptoms, and the system review was negative. Physical examination was within normal limits.

Review of the chest roentgenogram (figure 1) revealed a homogeneous, well-circumscribed, rounded density extending from the mediastinum into the right lung field. Lateral views (figure 2) indicated that the lesion arose in the posterior mediastinum. There was no definite involvement of lung parenchyma, the cardiovascular shadows were normal, and no relationship between the mass and the aorta was apparent.

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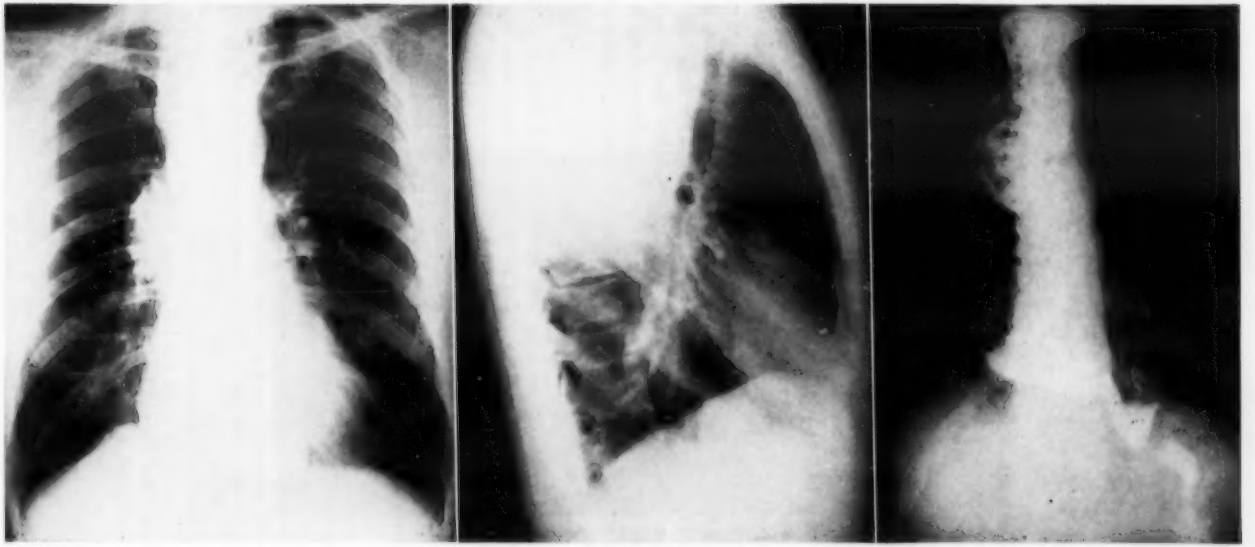


Case 1

FIG. 1 (Left). Admission chest roentgenogram demonstrates a rounded density projecting from mediastinum into right midlung field. Note also slight widening of the superior mediastinum and the crescentic air shadow at the level of the clavicle on the right.

FIG. 2 (Center). Lateral chest roentgenogram shows that lesion arises chiefly from the posterior mediastinum.

FIG. 3 (Right). Barium study defines mediastinal "mass" as dilated esophagus.

Because of the paucity of symptoms, benign neoplasm or a granulomatous process were the primary considerations, although lymphoma or other forms of malignancy remained distinct possibilities. Skin tests were applied, sputum samples obtained for cell study and culture, and arrangements were made for bronchoscopy and right scalene node biopsy. Prior to these latter procedures, additional chest roentgenograms were obtained to define more clearly certain relationships of the mass. These somewhat unexpectedly revealed an air-fluid level at the superior border of the "mass," and the diagnosis of achalasia was made. This impression was confirmed by a barium esophagogram (figure 3).

Renewed questioning of the patient now elicited a past history of postprandial regurgitation and mild epigastric distress. These symptoms had first been noticed four years earlier. A private physician had been consulted at that time who told the patient he had "cardiospasm" and placed him on a regimen of tincture of belladonna. The patient noted gradual disappearance of his symptoms, eventually discontinued his medication, and had remained totally asymptomatic during the preceding eighteen months. The patient was unaware of the meaning of the term cardiospasm. He had assumed that it was a closed chapter in his life and had not merited mention.

Comment: Despite the presence of a markedly distended esophagus, the patient's symptoms had been so mild that he failed to remember them on initial questioning. The lack of symptoms, however, did not lessen the urgency of establishing the nature of the apparent mediastinal mass. In retrospect, the original chest roentgenogram revealed a crescentic air shadow at the upper right border of the "mass" which should have suggested the diagnosis of achalasia. However, the roentgenographic picture was unusual in several respects: the esophageal shadow was homogeneous and dense, rather than "stippled" or "mottled," and presented as a single, rounded, apparently circumscribed projection into the right chest, rather than as a scalloped or smoothly curved border reaching from apex to base. The value of an early barium esophagogram in the evaluation of any mediastinal mass is emphasized by this case.

Case 2. Eli. K., a 46-year-old Negro male, was admitted to the District of Columbia General Hospital some twelve hours after sudden onset of a left hemiplegia. The history, as obtained from relatives, revealed that the patient had never before been hospitalized. They were unaware of any previous illnesses or complaints, and stated that the patient had been well and working regularly.

Physical examination revealed a semicomatose Negro male whose eyes were deviated to the right. Blood pressure was 190 systolic, 110 diastolic in mm. of mercury; pulse 72; temperature 97.6° F. Grade two hypertensive vascular changes were present in the fundi. The lungs were clear to examination. The heart size was within normal limits and auscultation was normal except for an accentuated second aortic sound. There was a flaccid paralysis of the left arm and leg. Babinski and Hoffman signs were present on the left.

Lumbar puncture revealed an opening pressure of 280 mm. of saline. The cerebrospinal fluid was grossly bloody and was xanthochromic after centrifugation. The electrocardiogram was normal. A portable chest film revealed several rounded shadows projecting from the mediastinum into both the right and left lung fields (figure 4).

Relationships were now postulated between the mediastinal "mass" and the patient's cerebral symptoms. A consultant from the Pulmonary Disease Division was asked to review the case, and noted that the mediastinal "masses" were not of uniform density, but appeared mottled. In addition, a crescentic air shadow was seen to limit the superior and lateral margin of the uppermost mass. Since the patient had already been intubated, a small amount of lipiodol was instilled through the esophageal tube, and roentgenograms were taken. These confirmed the diagnosis of achalasia.

Appropriate precautions were then instituted to prevent aspiration of esophageal contents. The patient's neurologic deficit improved rapidly. He was soon able to give a history

Case 2

FIG. 4 (Upper). Admission chest roentgenogram reveals mediastinal densities projecting into both hemithoraces. Again note the subtle air shadow surmounting the mass at the level of the clavicle on the right.

FIG. 5 (Lower). Barium study demonstrates changes of advanced achalasia.

in which he specifically denied any gastrointestinal symptoms, including regurgitation, eructation, and dysphagia. He had no history of respiratory infections.

After an appropriate period of convalescence, a barium esophagogram was performed which revealed extreme dilatation and tortuosity of the esophagus (figure 5). Esophagoscopy confirmed the great dilatation of the upper esophagus. While the extensive angula-

tions of the organ prevented passage of the instrument to the cardia, no lesion was seen in the area which could be examined safely. In view of the patient's lack of symptomatology and of his good nutritional status, it was decided that no surgical intervention was indicated at this time.

Comment: This patient again demonstrated the lack of symptoms which may be encountered in the face of tremendous esophageal dilatation. Furthermore, the unusual contour of this greatly angulated esophagus suggested that the patient's condition was due to a mediastinal malignant process which had involved the brain through metastasis. Such a gloomy prognostic situation was rather rapidly transformed when the diagnosis of achalasia was introduced. Esophagoscopy to exclude the presence of an intrinsic esophageal lesion is probably unnecessary in such cases as this, for several observers have indicated that dilatation of this extent is rare in instances of cicatricial or neoplastic obstruction of the esophagus, especially in the absence of well-defined symptoms (5–9).

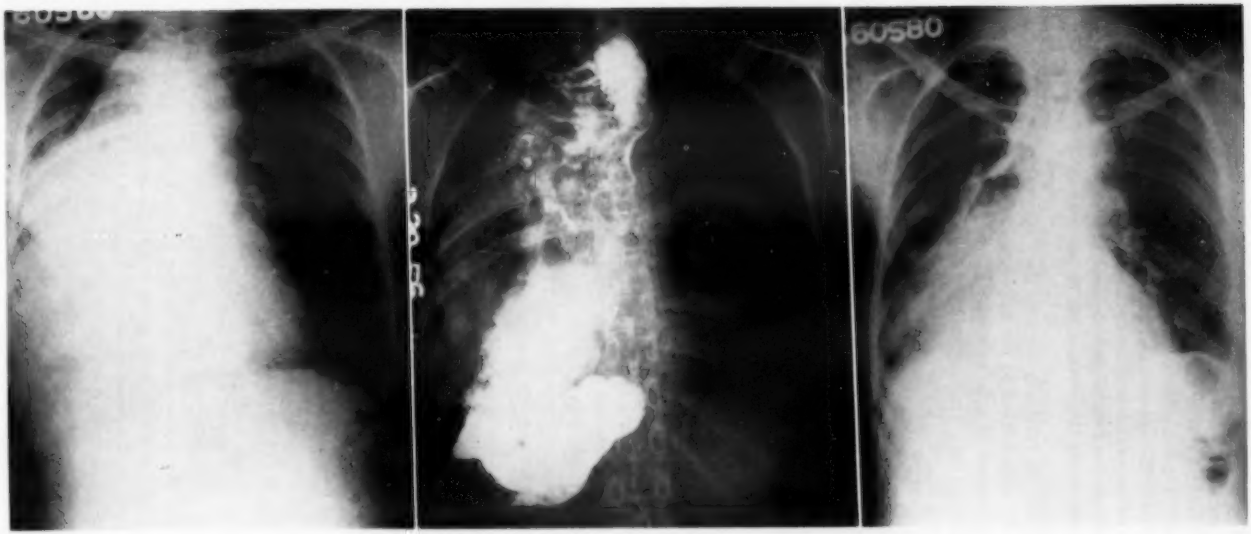
Case 3. S.H., a 68-year-old Negro female, was admitted to the Psychiatric Division of the hospital because of a depressive reaction which had culminated in a suicide attempt. The patient stated that she had "felt miserable" for at least six months, had little appetite, and had lost more than fifteen pounds. She had been greatly disturbed by financial problems and the recent death of a sister. The patient denied previous hospitalizations, but did recall that she had been advised to have a "goiter" removed some twenty years ago because of chronic dysphagia. She had refused operation and continued to have occasional difficulty in swallowing foods which she overcame by drinking large quantities of liquids with meals. She was also subject to considerable eructation and tolerated fatty foods poorly, both of these symptoms having been attributed to "gallbladder trouble."

Physical examination revealed a Negro female with evidence of recent weight loss who appeared markedly depressed. Her temperature was 100.5° F.; blood pressure, 130 systolic, 190 diastolic in mm. of mercury; pulse, 90. No thyroid tissue was palpable in the neck. There was no significant adenopathy. Posteriorly and laterally, the right chest was dull to percussion from apex to base, and breath sounds were absent. Anteriorly, bronchial breath sounds and scattered rales were present. Patchy rales were observed throughout the left chest. The remainder of the examination was unremarkable.

A chest roentgenogram was interpreted as follows: "A circumscribed density is present in the lateral portion of the right mid-lung field which is not well-defined . . . there appears to be atelectasis involving the right upper lobe. There is a pleural effusion at the right base. This may represent a bronchogenic carcinoma with atelectasis and pleural effusion; aneurysm should also be considered." (figure 6).

The patient was transferred to the Medical Service for evaluation. A diagnostic right thoracentesis was performed, and a small quantity of milky fluid was obtained. This was believed to indicate the presence of a chylothorax. The patient complained of no unusual distress during or following this procedure. The day following this procedure, the patient's roentgenograms were reviewed by the pulmonary disease consultant. A small crescentic air shadow was noted at the superior border of the lesion, and the diagnosis of achalasia was proposed. Therefore, a barium study of the esophagus was carried out. The radiologist reported: "Postero-anterior and right lateral chest films reveal a homogeneous density obscuring the right middle and lower lung fields and the medial half of the right upper lung field. After administration of a barium meal, it becomes evident that we are dealing with a huge and tortuous esophagus, partly filled with food residuals. The findings are consistent with achalasia of the esophagus" (figure 7). It was now recalled that the patient had been imbibing large quantities of milk prior to the thoracentesis which yielded "chylous fluid."

The patient was placed on a regimen of esophageal cleansing, following which esophagoscopy was performed. This further confirmed the diagnosis of achalasia, since no lesion of the esophagus was seen. A Heller procedure was then carried out which the patient tolerated extremely well.



Case 3

FIG. 6 (Left). Admission chest roentgenogram suggesting presence of neoplasm involving the mediastinum and right lung, with associated atelectasis and pleural effusion. Diagnostic air shadow can again be seen on the right at superior border of the "mass."

FIG. 7 (Center). Barium study demonstrates markedly dilated esophagus. Lateral portion of the esophagus is not filled with barium because of presence of residual food particles in this region.

FIG. 8 (Right). Postoperative chest roentgenogram reveals appreciable decrease in esophageal size.

Postoperatively, the esophagus decreased slightly in size (figure 8). Repeat barium studies showed normal transit from esophagus to stomach. The patient began to eat well and gained weight. Her mental status also improved, and she stated she had not felt so well for "many months." A psychiatric consultant re-evaluated the patient one month after operation and stated, "She appears much improved . . . not worried or depressed." The patient was discharged several days later to the care of her son. At her last clinic visit, three months after discharge, her mental and physical improvement had been maintained and she said that she could enjoy the foods of her choice without difficulty.

Comment: It is impossible to establish whether the "miserable feelings" and marked depression in this patient were related to the unpleasant symptoms she had apparently endured for a long time because of achalasia. Her marked improvement postoperatively suggests that such symptoms may have contributed to this mental state.

In retrospect, it appears that the history, physical findings, and roentgenograms should have defined the nature of the lesion rather clearly at the onset. However, it is common experience that the retrospective diagnosis of nonclassical instances of achalasia is usually obvious, while the original diagnostic approach is directed toward numerous other possibilities before the entity of achalasia is recalled. In this instance, for example, the presence of a roentgenogram suggesting mediastinal neoplasm with pleural effusion in an elderly patient with weight loss and anorexia could understandably lead to diagnostic neglect of cardiospasm. Such neglect can be eliminated if study of the esophagus with contrast material is accepted as an integral part of any thorough evaluation of mediastinal abnormalities.

General Comment

The cases described above indicate that cardiospasm may present in a variety of disguises. Such atypical presentations are far removed from the "classical" symptom-complex of dysphagia, foul eructation, regurgitation, and vague epigastric discomfort. Indeed, this "typical" picture is so frequently lacking that these patients are often misdiagnosed as suffering from such diverse disorders as psychoneurosis, pulmonary suppuration, and mediastinal neoplasm.

The relationship between cardiospasm and emotional disorders has been discussed by others (5–9). The problem continues to lack definition, chiefly because it represents a variation of the insoluble question of "which came first, the chicken or the egg?" *Case 3* provides further material for debate in this area.

In striking contrast is the unquestioned association of pulmonary infection with achalasia (10–13). Indeed, recurrent bouts of pneumonia or chronic bronchitic symptoms secondary to aspiration of esophageal contents may so predominate the clinical picture as to obscure the nature of the underlying disorder. Evaluation of the esophagus should not be neglected, therefore, in approaching patients with chronic or recurrent pulmonary infection.

By far the most foreboding disguise which cardiospasm may assume is that of a mediastinal neoplasm. A chest roentgenogram demonstrating mediastinal widening is always a source of concern to the physician. Such a finding immediately suggests a galaxy of pathologic possibilities, many of which have grim prognostic implications. The differential diagnosis, however, may omit consideration of achalasia as the cause of the abnormal roentgenographic shadow, since most discussions of mediastinal tumors neglect this entity (1–4, 14, 15). Appreciation of this diagnostic "pitfall," however, has been gradually increasing. In 1944, Hurst and Bassin (9) made the significant comment that "megacsophagus assumes an important position in the differential diagnosis of mediastinal ab-

normalities." Two of the eleven cases of achalasia presented by these writers had roentgenograms entirely compatible with the appearance of a mediastinal tumor. Seiler (16), in his classification of mediastinal tumors, recognized the problem and listed cardiospasm as a "pseudotumor" of the mediastinum. Perr (17) subsequently described in detail a case in point. In this patient, a young male, the diagnosis of mediastinal lymphoma had been made on the basis of serial roentgenograms. Some years before, he had received a discharge from the Army because of recurrent episodes of pneumonitis and persistent bronchitic symptoms. The diagnosis of cardiospasm was unexpectedly established by a barium swallow done to define relationships of the "tumor" as a prelude to definitive treatment.

Chandler (18) has described a similar case in which a 51-year-old white female presented with weight loss, anemia, fever, pleural effusion, and a "mediastinal mass." The diagnosis of a malignant mediastinal process seemed obvious. A barium swallow had been done previously and reported as normal. Fortunately, a repeat study was carried out as part of a pretreatment evaluation. A hugely dilated esophagus was found. After treatment by dilatation, the patient steadily improved, with weight gain, rise in blood count, and disappearance of the effusion. The same writer mentioned another instance known to him in which a patient with achalasia had received a course of deep X-ray therapy before the true nature of the condition was discovered.

Braun (19) has reported his experience with still another such case. The patient, a middle-aged white male, complained chiefly of recent weight loss. A chest roentgenogram revealed a multilobulated right mediastinal mass. It was of homogeneous density, and contained no air-fluid level. The diagnosis of a malignant neoplasm seemed justified on the basis of both history and roentgenographic findings. However, in the course of diagnostic evaluation, a barium swallow was done which unmasked the "mediastinal mass" as a dilated, tortuous esophagus.

Thus, achalasia is capable of producing not only an abnormal mediastinal shadow, but also a number of the signs and symptoms commonly associated with malignancy. Bleeding from the esophageal wall may lead to anemia. Poor nutrition, weight loss, and lack of appetite are not uncommon. Repeated episodes of aspiration may lead to chronic or recurrent bouts of fever and pneumonitis.

The ability of achalasia to masquerade as a mediastinal neoplasm is shared by esophageal diverticula (20, 21) as well as such lesions as eventration of the diaphragm, (22) diaphragmatic hernia, and dilatation of an azygos vein (23). The importance of being alert to such "sheep in wolf's clothing" is emphasized by the report of Levine and Greenfield (24). Reviewing a large series of "routine" chest films, those writers noted that 5 per cent of routine roentgenograms of a "healthy" population revealed some intrathoracic abnormality, and that asymptomatic mediastinal lesions were discovered once in every 2,000 such films. Considering the extent of modern roentgenographic screening programs, such lesions can no longer be considered as medical rarities. Equally significant is the writers' finding that "abnormalities of the esophagus and diaphragm" ranked fourth among causes of these abnormal mediastinal shadows, being more common than either developmental cysts or malignancies.

Recognition of atypical instances of cardiospasm is dependent upon three factors: a high index of suspicion, a careful history, and accurate roentgenographic interpretation. The cases reported in the present study demonstrate that the old medical adage "one must think of a disease to diagnose it" is especially applicable here. When faced with an abnormal mediastinal shadow, that cardiospasm may be present must first be considered. Second, a history of dysphagia and regurgitation must be sought carefully, since many

achalasia patients seem prone to neglect mention of such symptoms. Also a history of recurrent or chronic pulmonary infection should suggest the diagnosis of cardiospasm as a prominent consideration. Third, roentgenographic clues that define a "mediastinal mass" as a dilated esophagus should be kept in mind. As described by Katz (6), Hurst and Bassin (9), and others (12), these "clues" include:

(*1*) Diffuse widening of the mediastinum, which appears most marked on the right, since it is obscured by sternum and cardiac shadow on the left.

(*2*) An air-fluid level at the superior border of the apparent mass. As noted above, this pathognomonic sign can easily be overlooked. Moreover, as its presence depends upon the nature of the esophageal contents, an air-fluid level may be absent in achalasia when either air or fluid is not present. In such cases, a tell-tale crescentic "air-solid" shadow is often seen high in the thorax, representing air lying between the esophageal wall and the solid material filling the remainder of the lumen. In the writers' experience, this air meniscus had been an extremely valuable diagnostic sign. As noted in the case reports, its presence can lead to the diagnosis of achalasia in advance of barium studies.

(*3*) The widened mediastinal shadow is not uniformly dense, but is usually stippled or mottled due to the mixture of fluid, air, and solid particles in the esophagus.

(*4*) The gas bubble is usually absent from the stomach.

(*5*) The redundant esophagus may cause apparent widening of the right heart shadow and obliteration of the cardiophrenic angle.

(*6*) The "mass" usually extends from neck to diaphragm; however, marked lobulations may occur which are responsible for most instances of diagnostic confusion.

When historical and roentgenographic features indicate the presence of achalasia, the diagnosis can be readily confirmed by a barium esophagogram. Of even greater importance, however, is the recognition that, in instances in which the diagnosis is *not* suggested, cardiospasm will still be discovered if barium swallow has been incorporated routinely into the diagnostic evaluation of any mediastinal abnormality.

Achalasia is now amenable to rather simple corrective procedures in most instances (6-8). Early diagnosis is valuable, since advanced esophageal changes may render cases of long duration less responsive to corrective measures. Furthermore, elimination of the considerable danger of aspiration makes prompt diagnosis and treatment imperative.

As the physician encounters an increasing number of intrathoracic diagnostic problems through the intervention of mass roentgenographic techniques, it becomes essential to establish a thorough, orderly scheme of diagnostic evaluation. Adhering to such an approach will assure that such entities as achalasia will be detected, while thoracotomy will not be dangerously delayed in those instances which demand surgical intervention for diagnostic accuracy.

Summary

Three cases of achalasia are described in which the dilated esophagus suggested the presence of a mediastinal tumor roentgenographically and presented diagnostic difficulties. The clinical and roentgenographic features of achalasia are discussed. The occurrence of a crescentic air meniscus at the superior border of "abnormal mediastinal shadows" is stressed as a valuable diagnostic sign in achalasia. The importance of including barium swallow as part of the diagnostic evaluation of any mediastinal abnormality is also emphasized.

Sumario

Acalasia: Dolencia Capaz de Simular Neoplasia Mediastínica

Descríbense 3 casos de acalasia en los que la esofagectasia sugería radiográficamente la existencia de un tumor mediastínico y creó dificultades en el diagnóstico. Discútense las características clínicas y roentgenográficas de la acalasia. Como valioso signo diacrítico en ésta, recálcase la ocurrencia de un menisco semilunar de aire en el borde superior de las "sombras mediastínicas anormales." También se recalca la importancia de incluir la ingestión de bario como parte del estudio diagnóstico de toda anomalía del mediastino.

Resume

Achalasie: Phénomène apte à simuler une néoplasie médiastinale

Description de trois cas d'achalasie dans lesquels l'oesophage dilaté révélé par les radiogrammes suggèrait la présence d'une tumeur médiastinale, d'où la difficulté du diagnostic. Les aspects cliniques et radiologiques de l'achalasie sont étudiés. La présence d'un ménisque gazeux en forme de croissant à la limite supérieure d'une "opacité médiastinale anormale" est signalée comme un signe diagnostic important dans l'achalasie. L'importance de l'ingestion de baryum comme participation à l'évaluation diagnostique de toute anomalie médiastinale est également mise en évidence.

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Editorial

The Limitations of Our Knowledge About Para-Aminosalicylic Acid

Para-aminosalicylic acid (PAS) was found by Lehman to inhibit *M. tuberculosis* in 1943, and has been used to treat the disease since 1946.[1] After almost twelve years we still have very little knowledge of how it works. The available information raises more questions than it answers.

Para-aminosalicylic acid differs from isoniazid *in vitro* as follows: C^{14}-labeled isoniazid in culture medium is firmly bound to virulent tubercle bacilli grown upon it. The binding is easily broken by washing, however, when the bacilli used are isoniazid resistant. In direct contrast, C^{14}-labeled PAS is not bound by PAS-susceptible tubercle bacilli, but is bound to PAS-resistant bacilli.[2]

The combined effect of orally administered PAS with streptomycin in human tuberculosis is greater than one would have anticipated from the effectiveness of each drug separately. The PAS supplements the streptomycin, presumably because of the reduced chance of bacterial mutation to resistance to two drugs.[3, 4] This may be the main consideration since, clinically, PAS seems to act by prolonging rather than by augmenting the initial response to streptomycin.[5] The less obvious benefit of adding PAS to isoniazid therapy in human patients has been explained in a similar fashion. Judging from the much greater effectiveness of both streptomycin and isoniazid than PAS as single drugs, one would have anticipated that streptomycin would have proved quite superior to PAS as a companion drug to isoniazid in human tuberculosis. While numerous factors prevent completely reliable comparisons, it is reasonably clear that this is not the case, in so far as streptomycin twice weekly *versus* daily PAS is concerned.[6] The effectiveness of PAS with isoniazid may be explained partially by the elevation of the serum concentrations of biologically active isoniazid by the PAS in many patients. It has been suggested that this phenomenon can be explained, at least in part, through competition of the PAS with isoniazid for the acetylation capacity of the host.[7-11]

As is true of so many other chemotherapeutic agents, it is not known whether PAS should be administered orally so as to maintain constant serum concentra-

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tions and, if so, at what level, or whether one should try to achieve intermittent peaks of drug concentration. There is some indication that a dose of 5 or 6 gm. of sodium PAS daily is less effective than 10 to 12 gm. per day in association with 300 mg. of isoniazid per day, at least for the first four to eight months of therapy in humans.[12] Similar studies in association with streptomycin have shown that a dose of 10 to 12 gm. of sodium PAS by mouth is more effective than lesser amounts[13] and that, with streptomycin, 15 to 24 gm. of sodium PAS per day have only a slight therapeutic advantage over 12 gm. per day.[14] The administration of PAS intermittently with streptomycin twice weekly was distinctly less effective than when the PAS was given daily.[15] Oral PAS given in a single daily dose of 6 gm. was about as effective as twice as much given in three equal parts at one hundred and twenty days.[16]

Para-aminosalicylic acid is marketed in many different compounds, i.e., the acid, the sodium, calcium, calcium benzoyl, and potassium salts, and mixed with resins. These substances vary widely in their water solubilities, chemically determined concentrations attained in the blood,[17, 18] intestinal absorption, and patient toleration. The drug may be taken by patients in various physical forms, powder, compressed tablets, enteric-coated tablets, and granules and, most commonly, dissolved in water (either prepared in advance by the pharmacist or by the patient just before using).

In many European centers it has been standard practice to give 10 to 20 gm. of sodium PAS intravenously two to seven times a week for the first one to three months of treatment.[19, 20] The results are reported to be quite superior to those obtained with orally administered PAS, and this might be attributable to very high peak concentrations (24 to 45 mg. per 100 ml.) of biologically active PAS.[21, 22]

When PAS is administered by infusion, the drug concentrations measured biologically approximate those measured chemically in both blood and urine during the first three hours; in samples taken later, values obtained biologically are inferior to those measured chemically.[21] When given orally, peak concentrations of biologically active PAS are apt to be much lower than those measured chemically.[21] On the other hand, oral PAS in three daily doses of customary (4 to 5 gm.) size will maintain a concentration of 2 mg. of biologically active drug per 100 ml. for a longer period than will a single large (18 gm.) oral dose

[12] USPHS Cooperative Studies of the Chemotherapy of Tuberculosis: Unpublished data.

[13] Warner, O. M.: Tr. Eighth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1949, p. 112.

[14] Daniels, M. and Hill, A. B.: Brit. M. J., 1952, No. 4769, 1162.

[15] Hughes, F. J., Jr., *et al.*: Tr. Tenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1951, p. 67.

[16] Bridge, E. V., *et al.*: Tr. Twelfth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1953, p. 109.

[17] Riska, N.: Acta tuberc. Scandinav., 1955, *30*, 144.

[18] Cohen, R. V., *et al.*: Dis. of Chest, 1956, *30*, 418.

[19] Favez, G., *et al.*: Schweiz. Ztschr. Tuberk., 1956, *13*, 1.

[20] Jones, J. S.: Brit. J. Tuberc. 1954, *48*, 286.

[21] Schmiedel, A.: Centralbl. f. Bakt., 1955, *164*, 576.

[22] Lauener, H., *et al.*: Ztschr. f.d. ges. exper. Med. (in press).

or a rapid intravenous infusion.[22] Two milligrams of PAS per ml. of medium represent the approximate minimal inhibitory concentration *in vitro*. In large doses, rapid intravenous infusions are said to be adrenocorticotropic—a possible partial explanation of their effectiveness.[23]

In humans, PAS is excreted in several forms[24-26]; in one of these forms—acetyl PAS—there is blocking of the free amino group on the benzine ring, so that it is not measured chemically as the Lehman or Bratton-Marshall test is customarily performed, i.e., without acid hydrolysis.[27] The other major metabolite is para-aminosalicyluric acid (PAS-U, glycine conjugate of PAS or glycocoll-PAS). Two or three unidentified amines have also been described.[28] PAS-U and the free drug both retain the unblocked free amino group on the benzine ring and are therefore detectable by standard chemical methods.

In the few patients whose urinary output of PAS metabolites have been studied thoroughly so far,[22, 28] there was wide variation in the excretion of free PAS, acetyl PAS, and PAS-U from person to person. The amount of PAS-U excreted by individuals is reported to rise with the passage of time.[29] This may be important since this metabolite, like acetyl PAS, is essentially inert biologically.[21, 22, 30-32] It is not yet known whether there is a close correlation in individuals between urinary excretion and serum concentrations of free, biologically active PAS and its metabolites. It is also not known whether those subjects who inactivate isoniazid excessively are the ones who handle PAS in a similar manner, although preliminary information suggests that this is the case.[33] It is quite likely that studies of this kind may explain in some measure some failures of streptomycin-PAS as well as isoniazid-PAS therapy.

These comments with regard to the biologic activity of PAS are made with some hesitation, since the measurement of the antituberculous activity of PAS *in vivo*[34] and *in vitro*[21, 32, 35-37] is bound to be uncertain at best. The compound is an unstable one, particularly in water solution.[38] Some of the breakdown products of PAS, e.g., phenolic, may have as much or more antituberculous activity *in vitro* than the parent substance. The drug in body fluids may undergo changes significantly affecting biologic activity between the time the specimen is ob-

[22] Favez, G., *et al.*: Dis. of Chest, 1954, *26*, 646.
[24] Way, E. L., *et al.*: J. Pharmacol. & Exper. Therap., 1948, *93*, 368.
[25] Venkataraman, A., *et al.*: J. Biol. Chem., 1948, *173*, 641.
[26] Kawamata, J., and Kashiwagi, K.: Med. J. Osaka, 1956, *6*, 119.
[27] Lehman, J.: Scandinav. J. Clin. & Lab. Investigation, 1952, *4*, 142.
[28] Way, E. L., *et al.*: J. Am. Pharm. A., 1955, *44*, 65.
[29] Zini, F.: Rev. Brit. Clin. Med., 1953, *53*, 308.
[30] Lauener, H., and Dettwiler, E.: Helv. physiol. pharmacol. Acta, 1953, *11*, C58.
[31] Oberweger, K. H., *et al.*: Quart. J. Pharm. & Pharmacol., 1948, *21*, 292.
[32] Goodacre, C. L. *et al.*: Quart. J. Pharm. & Pharmacol., 1948, *21*, 301.
[33] Bell, J. C., and Mitchell, R. S.: Unpublished data.
[34] Youmans, G. P. *et al.*: J. Bact., 1947, *54*, 409.
[35] Carr, D. T., *et al.*: Proc. Staff Meet., Mayo Clin., 1952, *27*, 209.
[36] Morse, W. C.: Unpublished data.
[37] Middlebrook, G.: Unpublished data.
[38] Schoenholzer, G.: Unpublished data.

tained from the patient and the *in vitro* test. Since the *in vitro* antituberculous activity of PAS is rather mild, a reading of PAS activity must be based upon relative inhibition or colony size rather than upon complete suppression of bacterial growth, as in the case of streptomycin or isoniazid. The selection of media and inoculum size appears to play an important role in these tests of PAS susceptibility. In short, the antituberculous activity of PAS and its metabolites should be reappraised, preferably with a more standardized and reliable method than those utilized up to this time. The chemical measurement of free PAS in body fluids is relatively easy to perform and is reliable; it should therefore be a good measure of biologic activity unless there are considerable differences in concentration of the drug between serum, tissues, and urine, and frequent shifts back and forth between free PAS and its metabolites.

The toxicity of PAS has also posed difficult problems, not only in the clinic but also in the laboratory. The dangerous forms of toxicity fortunately occur quite infrequently and consist of true PAS hypersensitivity[39] and severe hepatic[40, 41] and renal[41] damage. Gastrointestinal intolerance, with nausea, vomiting, abdominal pain, and diarrhea, is quite common, more common with advancing years and in the severely ill. It has been the main cause for discontinuation of PAS therapy in 10 to 70 per cent of patients.[42] This wide variation in tolerability of PAS can only be explained by varying attitudes of physicians toward the relative importance of PAS compared to other available agents; and by a variation in the quality of the PAS[43] which reaches the patient's gastrointestinal tract. There is little doubt that the practice of dissolving PAS in water, except immediately before it is to be ingested, is one preventable cause of intolerance.

Intolerance to PAS is apparently related to its degree of discoloration in concentrated water solution.[43] Yet meta-aminophenol, long thought to be the principal toxic contaminant of commercial PAS, is quite colorless. While meta-aminophenol is toxic for humans in large doses,[22] the addition of 4 per cent of the compound to pure fresh PAS did not materially increase the toxicity of PAS in humans.[44]

The discoloration of PAS appears to be potentiated by moisture, heat, ultraviolet irradiation, contact with heavy metals, and a low pH.[37, 45] The passage of time increases the chance that any or all of these factors may enter the picture. The cause of the discoloration is probably polymerization,[38] not only of PAS with PAS, but also of PAS with meta-aminophenol. Oxidation of the meta-amino-

[39] Sandler, A.: Brit. J. Tuberc., 1955, *49*, 231.

[40] Bellamy, W. E., *et al.*: Ann. Int. Med., 1956, *44*, 764.

[41] Jones, J.: Unpublished data.

[42] VA-Armed Forces Cooperative Studies of Chemotherapy of Tuberculosis: Unpublished data.

[43] Mitchell, R. S.: J. Chronic Dis., 1956, *4*, 607.

[44] Mitchell, R. S., *et al.*: J.A.M.A., 1954, *156*, 124.

[45] Mitchell, R. S., *et al.*: Clinical and biochemical studies of PAS utilization and intolerance (unpublished data).

phenol-PAS polymer is also a probable factor in the deterioration and discoloration process.[25]

The speed with which freshly prepared PAS will deteriorate varies tremendously, depending upon the quality of its manufacture, packaging, shipping, storage, and the manner of its preparation for patient consumption. Freedom of PAS from meta-aminophenol in manufacture is also important since prolonged contact with the latter compound potentiates the deterioration of PAS both by polymerization and decarboxylation.[25, 38]

The toxicity and intolerance to PAS appear to be significantly less by the intravenous than by the oral route.[19-21] It is possible that oral PAS is altered by stomach and intestinal passage into substances of reduced biologic activity and/or increased toxicity for humans. Preparations of PAS labeled with C^{14} in the carboxyl group were decarboxylated into meta-aminophenol in the stomach of guinea pigs, as 4 per cent of the labeled carbon was recovered as $C^{14}O_2$ in the expired air. When the same substance was placed in the intestine below the ligated pylorus, less than 1 per cent was so recovered.[46] While decarboxylation of PAS in the human stomach is only one-third of that in the guinea pig's stomach,[46] there may prove to be some relationship between the degree of individual stomach acidity and PAS intolerance. In clinical practice it appears likely that intolerance originates from something which happens in the gastrointestinal tract rather than after absorption into the blood stream.

It has been found that PAS can be conjugated into compounds of beautiful crystalline structure, pure white color, and greatly increased stability by a number of organic acids.[47] One of these—a vitamin C conjugate—has been tried in humans with fair toleration and high sustained drug concentrations in the blood.[45, 48] Vitamin C has long been known to act as a preservative of the white color of PAS, probably because it is a mild reducing agent.[49] Moreover, the administration of PAS to mice and to humans appears to increase their Vitamin C requirements.[47]

The symptoms of human intolerance to PAS can be reduced by the simultaneous administration of various adsorbing and antacid agents, e.g., magnesium trisilicate, aluminum hydroxide, and kaopectate. Unfortunately, these agents also interfere in some way with absorption, since the serum concentrations and the urinary excretion of PAS are thereby reduced. Since only traces of PAS are found in the stool, these adjuvants also appear to promote a major breakdown of the PAS molecule in the gastrointestinal tract,[45] probably by delaying the rate of absorption.

The quality of the commercial drug is not well controlled at present. For instance, the U.S. Pharmacopeia (XV) permits a faint yellow color of the commercial drug in the dry state. Preparations of PAS which are faintly yellow in

[46] Heller, A., *et al.*: Am. Rev. Tuberc., 1957, *75*, 71.
[47] Scheel, L.: Unpublished data.
[48] Browning, R. H.: Unpublished data.
[49] U.S. Patent #2,552,486, American Cyanamid Co., May 8, 1951.

the dry state may appear quite brown in concentrated solution. While it is possible to restore the white color of yellow PAS with reducing agents, any effect this may have on tolerability and effectiveness *in vivo* is not clear.

Numerous questions are in need of an answer. What is the best form of the drug to use? When should it be administered orally and when by the intravenous route? Should dosage be based upon body weight, upon drug concentrations in the blood, or upon the selection of the companion drug? How often should PAS be given daily? Is there a correlation between the degree of brownish-purple discoloration of PAS in concentrated water solution, on the one hand, and *biologic* evidence of deterioration on the other? How much of this discoloration, if any, should be permissible in commercially available PAS? Using methods which distinguish between biologically active and inactive PAS in serum and urine, will there be a good correlation between these biologic concentrations and the clinical effectiveness of PAS? Do individuals inactivate PAS to the same degree as they inactivate isoniazid, which is given in proportionately so much smaller doses? Does the pattern of PAS inactivation change with the passage of time? The emergence of PAS-resistance is apparently more delayed and less frequent than is resistance to streptomycin and isoniazid. Does this reflect inadequate delivery of biologically active PAS to the infecting organisms in many patients? Inhibition by what minimal concentration of PAS *in vitro*[50, 51, 52] is the most nearly correct measure of susceptibility of tubercle bacilli *in vivo*?

In conclusion, PAS is a valuable but poorly understood and controlled drug used in the therapy of tuberculosis. While notable improvements have been made in its preparation and use in recent years, a plea is made for more investigation in this important and controversial field.

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[50] MITCHISON, D. A.: Brit. M. Bull., 1954, *10*, 115.

[51] RUSSELL, W. F. JR., *et al.*: Chemotherapy of Tuberculosis in *Advances in Internal Medicine*, Year Book Publishers, Inc., Chicago, 1956.

[52] RIST, N.: Unpublished data.

Notes

DRUG SUSCEPTIBILITIES OF 20 "ATYPICAL" AS COMPARED WITH 19 SELECTED STRAINS OF MYCOBACTERIA[1,2]

The increasingly frequent recognition of cases of pulmonary disease associated with mycobacteria which are nonvirulent for guinea pigs and commonly pigmented has stimulated an intensive effort to determine the relationship of these so-called atypical strains to other mycobacteria. Runyon[3] tentatively has divided the large collection of these strains in the National Tuberculosis Association-Veterans Administration depot[4] into three main groups according to their pigmentation: *photochromogens* (yellow only upon exposure to light); *nonphotochromogens* (white, cream, or yellow, but pigmentation not light-conditioned); and *skotochromogens* (yellow-orange even in complete darkness). The present study was undertaken to see whether the pattern of drug susceptibility of the atypical strains might aid in their grouping and classification and to obtain leads for chemotherapy trials, since disease associated with these strains does not respond uniformly to the currently used antituberculous agents. The trial was part of a comprehensive coordinated investigation sponsored by the Laboratory Subcommittee of the American Trudeau Society.

A list of the mycobacterial strains used and the source of each is given in table 1. All but 3 of the atypical strains had been recovered repeatedly from the sputum of a patient with pulmonary disease and/or from the lung tissue itself, and, with only one exception, in the absence of typical tubercle bacilli.

None could produce progressive disease when inoculated subcutaneously into guinea pigs, and the majority had been isolated before the patient had received drug therapy. Data concerning the drugs are presented in table 2. The first nine drugs may be classified as antituberculous agents *in vitro*, the next six as antimicrobial drugs active primarily against organisms other than mycobacteria, and the last seven as antifungal agents.[5]

Stock solutions of the drugs were freshly prepared, sterilized by filtration, and added in the required amounts to the egg medium before and 80°C.-one hour inspissation and to the melted agar medium at 56°C. The tests were performed within a day or two after the media were slanted and solidified in tubes. Agar medium was used for drugs liable to deteriorate during inspissation. The cultures were grown in Tween®-albumin medium for one or two generations, then inoculated onto the test media with a loop. Each culture was planted in duplicate, the first series with inoculum adjusted to give a moderately heavy but not confluent growth on the control medium, and the second series with a tenfold

[1] Presented in part before The Medical Session, as part of *Section 2B*, at the annual meeting of The National Tuberculosis Association, Kansas City, Missouri, May 6, 1957.

[2] This study was aided in part by a grant, E36(C10), from the National Institutes of Health, Public Health Service.

[3] Runyon, E. H.: Am. Rev. Tuberc., 1955, *72*, 866.

[4] Culture depot at Sunmount, New York sponsored jointly by the National Tuberculosis Association and the Veterans Administration.

[5] Drugs were generously supplied as follows: isoniazid, streptomycin, Pas, viomycin, neomycin, oxytetracycline, and rimocidin® by Chas. Pfizer & Co.; nystatin and MC 3277 by E.R. Squibb & Sons; filipin and actidione by The Upjohn Co.; pyrazinamide and stilbamidine by Merck & Co.; SU 1906 by Ciba Pharmaceutical Products; cycloserine by Eli Lilly & Co.; erythromycin by Abbott Lab.; asterol by Hoffman-LaRoche; and amithiozone by Nepera Chem. Co.

TABLE 1

LIST AND SOURCE OF THE 29 STRAINS OF MYCOBACTERIA

Group	Strain	Source
Atypicals—20	Photochromogens—9 Nonphotochromogens—7 Skotochromogens—4	NTA—VA* depot (Runyon) at Sunmount, N. Y., through cooperative study sponsored by ATS Laboratory Subcommittee
M. tuberculosis var. *hominis*—6	Virulent: H37Rv Campbell Erdman Recent isolate from untreated patient Attenuated: R1Rv Avirulent: H37Ra	 NTA Culture Bank† NTA Culture Bank NTA Culture Bank Trudeau Laboratory NTA Culture Bank NTA Culture Bank
M. tuberculosis var. *bovis*—4	Ravenel Ravenel resistant to isoniazid 4228-3 4228-4	NTA Culture Bank NTA Culture Bank NTA Culture Bank NTA Culture Bank
M. avium—3	Sheard Kirchberg A_1	NTA Culture Bank NTA Culture Bank NTA Culture Bank (old laboratory strain)
Rapidly growing saprophytes—3	*M. phlei* *M. ranae* *M. fortuitum* (Aguis)	NTA Culture Bank NTA Culture Bank A. Q. Wells, through R. J. Dubos
Miscellaneous—3	*M. tuberculosis* var. *muris* OV 183 (vole bacillus) *M. balnei*, strain V *M. ulcerans* KR	NTA Culture Bank from A. Q. Wells, Oxford ATCC 11567, through Linell; Zettergren NTA Culture Bank, from P. MacCallum, Melbourne

* See footnote 3.

† National Tuberculosis Association standard culture depot maintained at the Trudeau Laboratory.

dilution. For all nonaqueous stock solutions the control slants contained an amount of the solvent equal to that in the highest concentration of drug. Final readings of growth were made after two to six weeks of incubation. Evaluation of drug susceptibility was based on a comparison of the amount and time of appearance of growth in the drug and control tubes. "Susceptible" indicates that there was no significant growth on the lowest drug concentration; and "resistant," equivalent growth on the highest drug concentration and on the control. The terms "slightly" and "moderately" resistant indicate growth in the low and medium drug concentrations, respectively, while those strains that grew in the

TABLE 2

THE 22 ANTIBACTERIAL AND ANTIFUNGAL DRUGS

Drug	Solvent	Medium	Concentrations in γ or Units per Ml	Reference Footnote
Isoniazid	Water	S and S*	0.1, 1.0, 10, 50	
Streptomycin	Water	7H-9†	2, 10, 50, 200	
PAS	Water	S and S	1, 10, 100	
Pyrazinamide	Water	S and S pH 5.3	10, 25, 100, 500	7
Viomycin	Water	7H-9	10, 50, 200	
Neomycin	Water	7H-9	1, 10, 100 (units)	
Cycloserine	Water	S and S	20, 40, 100, 500	
Amithiozone	Propylene glycol	S and S	5, 10, 50	
Thiocarbanilide SU 1906	Dimethylacetamide	S and S	5, 10, 25, 100	8
Oxytetracycline	Water	7H-9	20, 100, 500	
Chloramphenicol	Water	7H-9	10, 50, 200	
Erythromycin	Alcohol	7H-9	1, 10, 100	
Penicillin G	Water	7H-9	10, 100 (units)	
Bacitracin	Water	7H-9	100	
Polymyxin B	Water	7H-9	80	
Stilbamidine	Water	7H-9	10, 100	
Pyridinethione MC 3277	Water	7H-9	0.1, 2.0	9
Filipin	Alcohol	7H-9	10, 100	10
Nystatin	Dimethylacetamide	7H-9	10, 100 (units)	11
Rimocidin	Dimethylacetamide	7H-9	10, 100	12
Actidione	Water	S and S	10, 200	13
Asterol (Benzothiazole)	Water	7H-9	20, 500	14

* Inspissated egg medium (STEENKEN, W. JR., AND SMITH, M. M.: Am. Rev. Tuberc., 1938, *38*, 514.)

† Middlebrook's modification of oleic-acid–albumin agar (Cohn *et al.*: Am. Rev. Tuberc., 1954, *70*, 465; Middlebrook *et al.*: Am. Rev. Tuberc., 1954, *70*, 852.

first two tubes of a three-tube test were considered to show "intermediate" resistance. All of the readings and evaluations were carried out by one of the writers without knowledge of the identity of the strains.

A summary of the results obtained with some of the drugs is presented in table 3. The atypical strains as a group were more resistant than *M. tuberculosis* to all of the antituberculous agents except cycloserine. A similar situation was noted with oxytetracycline and chloramphenicol, but the atypical mycobacteria were slightly more susceptible than tubercle bacilli to erythromycin. No significant differences were observed in the susceptibilities of the various groups of mycobacteria to penicillin, bacitracin, or polymyxin B. Of the 7 antifungal agents, 6 showed no appreciable activity. Some of the atypical strains were slightly more susceptible to stilbamidine than were the *M. tuberculosis* strains, and the pyridinethione compound inhibited most of the cultures in concentration of 2 γ per ml.

Particularly interesting were the patterns of activity exhibited by amithiozone and the

TABLE 3

IN VITRO DRUG SUSCEPTIBILITIES OF 39 STRAINS OF MYCOBACTERIA

Figures Represent Number of Strains

	Isoniazid				Streptomycin				PAS			Pyrazinamide				Viomycin			Neomycin			Cycloserine			Amithiozone			Thiocarbinilide SU 1906				Oxytetracycline				Chloramphenicol			
	S	Sl	M	R	S	Sl	M	R	S	Int	R	S	Sl	M	R	S	Int	R	S	Int	R	S	Int	R	S	Int	R	S	Sl	M	R	S	Sl	M	R	S	Sl	M	R
Atypicals																																						
Photochromogens—9	0	8	1	0	0	6	3	0	0	8	1	0	0	7	2	0	9	0	0	9	0	9	0	0	9	0	0	7	2	0	0	0	1	8	0	0	3	6	0
Nonphotochromogene—7	0	3	4	0	0	2	4	1	0	2	5	0	0	0	7	0	7	0	0	6	1	7	0	0	1	6	0	0	2	0	5	0	0	6	1	0	0	3	4
Skotochromogene—4	0	0	4	0	0	2	2	0	0	4	0	0	0	1	3	2	2	0	2	2	0	4	0	0	0	4	0	0	0	0	4	1	1	2	0	1	1	0	2
M. Tuberculosis var. *hominis*																																						
Virulent—4	4	0	0	0	4	0	0	0	4	0	0	4	0	0	0	4	0	0	4	0	0	4	0	0	4	0	0	2	2	0	0	1	3	0	0	3	1	0	0
Nonvirulent—2	2	0	0	0	2	0	0	0	2	0	0	0	2	0	0	1	1	0	2	0	0	2	0	0	2	0	0	2	0	0	0	1	1	0	0	2	0	0	0
M. Tuberculosis var. *bovis*—4	3	0	0	1*	4	0	0	0	2	2	0	0	0	0	4	4	0	0	4	0	0	3	1	0	4	0	0	1	3	0	0	3	1	0	0	4	0	0	0
M. avium—3	0	0	3	0	0	3	0	0	0	3	0	0	0	2	1	0	3	0	0	3	0	3	0	0	0	3	0	0	0	1	2	0	1	2	0	0	1	1	1
Saprophytes—3	0	0	3	0	2	0	1	0	0	0	3	0	0	0	3	1	1	1	2	1	0	1	2	0	1	2	0	0	0	3	0	0	0	0	3	1	1	1	0
M. Tuberculosis var. *muris*	1				1					1			1			1			1			1			1				1				1			1			
M. balnei			1			1				1					1		1			1		1			1		1						1				1	
M. ulcerans				1		—				—			—				—			—		1			1				—				—				—	

— Dashes indicate test unsatisfactory because of poor growth.

* This strain was the Ravenel which had been made resistant to isoniazid *in vitro*.

Abbreviations: S—susceptible; Sl—slightly resistant; M—moderately resistant; Int—intermediate or partial resistance; R—resistant to highest concentration of drug used; PAS—*p*-aminosalicylic acid.

thiocarbanilide compound. The photochromogenic atypical and the *M. tuberculosis* cultures were clearly more susceptible than the other two groups of atypical strains and the saprophytes to these drugs. Cross resistance between the two drugs has been noted previously.[6] Also worthy of note was the observation that all of the mycobacterial strains tested except *M. tuberculosis* var. *hominis* and var. *muris* were resistant to pyrazinamide in acidified media. In addition, pyrazinamide has been found to be ineffective in mice infected with a photochromogenic atypical strain or with a bovine tubercle bacillus, in contrast to its good activity against infections produced by human strains of *M. tuberculosis*.[7]

The avian strains were usually more drug resistant than *M. tuberculosis* except with respect to stilbamidine (to which they were more susceptible) and cycloserine and pyridinethione (to which they were equally susceptible). The vole bacillus tended to behave like the *M. tuberculosis* strains, while the photochromogenic *M. balnei* behaved more like the saprophytes in patterns of drug susceptibility. One skotochrome (National Tuberculosis Association-Veterans Administration depot package No. 15) was uniformly susceptible to all 6 of the antimicrobial agents active primarily against microorganisms other than tubercle bacilli. The significance of this observation is unknown.

* * *

The chief value of a study such as this lies in the comparison of drug susceptibilities among the different groups of mycobacteria rather than in the minimal inhibiting concentration of each drug for each strain, which will vary considerably depending on the test conditions. Thus, Tarshis,[8] employing other methods, found that 10 chromogenic strains of mycobacteria were resistant to 100 γ of isoniazid per ml., a level higher than that exhibited by the presently reported strains.

Most of the drugs showed no significant difference in activity against any of the three pigment groups of atypical strains. There was, however, a clear distinction in susceptibility to isoniazid and to the thiosemicarbazone-thiocarbanilide compounds between the photochromogens, on the one hand, and the saprophytes and the other two groups of atypical cultures, on the other. In this respect, the photochromogens behaved more like the *M. tuberculosis* strains, which may indicate a metabolic process distinct from that of the other groups.

Various combinations of cycloserine, amithiozone, thiocarbanilides, isoniazid, and streptomycin appear to be worthy of further animal and human trials in infections produced by atypical mycobacteria.[15]

In summary: A comparison has been made of the *in vitro* drug susceptibilities of 20 atypical and 19 selected strains of mycobacteria. The atypical strains chosen were considered to be significant in the pathogenesis of human pulmonary disease, and included

[6] Konopka, E. A., *et al.*: Proc. Soc. Exper. Biol. & Med., 1955, *89*, 388.
[7] Steenken, W., Jr., *et al.*: Am. Rev. Tuberc. (in press).
[8] Tarshis, M. C., *et al.*: Am. Rev. Tuberc., 1955, *72*, 119.
[9] Pansy, F. E., *et al.*: Proc. Soc. Exper. Biol. & Med., 1953, *82*, 122.
[10] Whitfield, G. B., *et al.*: J. Am. Chem. Soc., 1955, *77*, 4799.
[11] Hazen, E. L., and Brown, R.: Proc. Soc. Exper. Biol. & Med., 1951, *76*, 93.
[12] Davisson, J. W., *et al.*: Antibiot. & Chemother., 1951, *1*, 289.
[13] Whiffen, A. J., J. Bact., 1948, *56*, 283.
[14] Grunberg, E., *et al.*: Tr. New York Acad. Sc., 1950, *13*, 22.
[15] Since this manuscript was written, the results of a preliminary trial of chemotherapy in mice has been presented (Steenken, W., Jr., *et al.*: Tr. Sixteenth Conference on Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1957 (in press)).

representatives of the three pigment types, photochromogens, nonphotochromogens, and skotochromogens. The drugs included 9 antituberculous agents, 6 general antimicrobials, and 7 antifungal agents. Although the atypical strains were generally more resistant than *M. tuberculosis* to the antituberculous drugs, they were equally susceptible to cycloserine. None of the general antimicrobials or antifungal agents were significantly active. The photochromogenic and the *M. tuberculosis* strains were clearly more susceptible to isoniazid, amithiozone, and to a thiocarbanilide than the other two groups of atypical strains and the saprophytes.

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SCALENE LYMPHADENOPATHY

A Post-Mortem Study

In 1949, Daniels[1] first called attention to the value of biopsy of the scalene lymph nodes in the diagnostic study of patients with various intrathoracic diseases, even for those patients without palpable lymph nodes. Since then many others[2-14] have reported their experience with this procedure and certain variations of it. All have agreed that it is of value, especially in the diagnosis of sarcoidosis.

It has been assumed generally that any disease in the lymph nodes is the same as the intrathoracic disease. However, Bosman and associates[3] mentioned the possibility that a tuberculous lymph node might be unrelated to the pulmonary disease, and Seghers and associates[12] reported finding a tuberculous scalene lymph node in a case in which further observation revealed the pulmonary lesion to be a carcinoma. Shefts and co-workers[13] also pointed out that correlation might not be complete between the disease in the mediastinum and that in the scalene nodes, and mentioned that a post-mortem study was in progress.

Biopsy of impalpable scalene lymph nodes has been performed for several years at the Mayo Clinic[15] in certain cases of undiagnosed intrathoracic disease, with results comparable with those reported by others. The assumption that the scalene lymphadenopathy was the same as the intrathoracic disease was questioned and, therefore, study of post-mortem material was planned in the hope that it would shed some light on the problem.

During part of 1954, both the right and the left scalene fat pads were removed at the time of 74 post-mortem examinations. Absence of palpable lymph nodes above the clavicles was the only criterion used in selection of these cases. In 4 cases no lymph nodes were found in the fat pads. Nodes were found in both pads in 25 cases, on the right side only in 16 cases, and on the left side only in 29 cases. The number of nodes found in the fat pads varied from one to four. The nodes were fixed in formalin, and microscopic sections were stained with hematoxylin and eosin. These sections were studied as unknowns by one of us (W.A.B.), and the diagnosis was made. Then these diagnoses were correlated with the intrathoracic diseases found to be present at the time of the post-mortem examinations.

In 52 of the 70 cases in which nodes were available for study, there was either no intrathoracic disease or irrelevant disease, such as terminal bronchopneumonia. Lymph nodes were available for examination from both sides in 16 of these cases; from the right side

[1] Daniels, A. C.: Dis. of Chest, 1949, *16*, 360.
[2] Baer, L. S.: Stanford M. Bull., 1952, *10*, 104.
[3] Bosman, G., *et al.*: Nederl. tijdschr. geneesk., 1951, *95*, 3382.
[4] Carstensen, B., *et al.*: Dis. of Chest, 1954, *25*, 443.
[5] Connar, R. C.: Surg., Gynec. & Obst., 1955, *101*, 733.
[6] Correll, N., Jr., and Langston, H. T.: Illinois M. J., 1956, *109*, 117.
[7] Cuykendall, J. H.: J.A.M.A., 1954, *155*, 741.
[8] Harken, D. E., *et al.*: New England J. Med., 1954, *251*, 1041.
[9] Kenney, L. J., and Mota, C.: Henry Ford Hosp. M. Bull., 1956, *4*, 36.
[10] Odelberg, A.: Ann. chir. et gynaec. Fenniae, 1954, *43* (Supplement 5) 272.
[11] Radner, S.: Acta med. Scandinav., 1955, *152*, 413.
[12] Seghers, K. K. M. F., *et al.*: Arch. chir. neerl., 1954, *6*, 57.
[13] Shefts, L. M., *et al.*: Am. Rev. Tubere., 1953, *68*, 505.
[14] Yang, W. W.: California Med., 1956, *85*, 165.
[15] Beahrs, O. H., *et al.*: Minnesota Med., 1957, *40*, 152.

TABLE 1

SIX CASES OF INTRATHORACIC DISEASE AND NEGATIVE SCALENE LYMPH NODES

Case	Intrathoracic Disease	Scalene Nodes	
		Right	Left
1	Miliary tuberculosis	No nodes	Negative
2	Pulmonary tuberculosis	Negative	Negative
3	Hodgkin's disease	No nodes	Negative
4	Pulmonary granulomatosis, cause undetermined	Negative	No nodes
5	Pulmonary granulomatosis, cause undetermined	No nodes	Negative
6	Pulmonary granulomatosis, cause undetermined	Negative	No nodes

TABLE 2

TWELVE CASES OF INTRATHORACIC DISEASE WITH SCALENE LYMPH NODES GIVING EVIDENCE OF SAME DISEASE

Case	Intrathoracic Disease	Scalene Nodes	
		Right	Left
7	Sarcoidosis	Positive	Negative
8	Adenocarcinoma of bronchus	Positive	Positive
9	Metastatic carcinoma from breast	No nodes	Positive
10	Metastatic carcinoma from breast	Positive	Positive
11	Metastatic carcinoma from pancreas	Positive	Positive
12	Hodgkin's disease	Positive	Negative
13	Hodgkin's disease	Positive	No nodes
14	Acute leukemia	No nodes	Positive
15	Acute leukemia	Positive	Positive
16	Acute leukemia	Positive	Negative
17	Acute leukemia	Positive	No nodes
18	Chronic lymphatic leukemia	Negative	Positive

only in 12 cases, and from the left side only in 24 cases. In all of these cases the lymph nodes were normal or showed such nonspecific changes as chronic lymphadenitis. In 2 of the cases in which cirrhosis of the liver was present, with obstructive jaundice in one, unusual phagocytosis was taking place in the lymph nodes.

In 6 cases in which pulmonary or mediastinal disease such as tuberculosis, granuloma of unknown etiology, or Hodgkin's disease was present, the scalene lymph nodes revealed no disease (table 1).

In 12 cases in which significant intrathoracic disease was present, this was evident also on examination of the scalene nodes on one or both sides (table 2).

* * *

This study confirms previous reports of the value of biopsy of impalpable scalene lymph nodes in the diagnosis of intrathoracic disease. Examination of the scalene lymph nodes revealed evidence of the intrathoracic disease in 12 of the 18 cases of such disease.

In no case did examination of the scalene nodes suggest a diagnosis which was not confirmed by the rest of the post-mortem study.

THOMAS D. LEE HOSPITAL
OGDEN, UTAH

WARREN A. BENNETT

THE MAYO CLINIC AND THE FOUNDATION, part of
GRADUATE SCHOOL OF THE UNIVERSITY
OF MINNESOTA
ROCHESTER, MINNESOTA

DAVID T. CARR

April 12, 1957

Correspondence

TREATMENT OF ACTIVE PULMONARY TUBERCULOSIS OUTSIDE INSTITUTIONS

To the Editor of The American Review of Tuberculosis and Pulmonary Diseases:

In your excellent magazine last November I found an especially interesting article entitled "Will History Repeat Itself?" by Henry Stuart Willis. I should like to add a few comments on the dangers of treatment of active pulmonary tuberculosis outside institutions.

In Korea, of course, the problem of tuberculosis is overwhelming, and I believe the increasing incidence would be startling if figures were available. The shift of population and hardships of malnutrition, exposure to weather, crowding, and lack of sanitation brought on by the war resulted in the expected increase in tuberculosis. However, the timely advent of isoniazid in 1952 helped tremendously to stem the tide. Through 1953 and 1954 results seemed to border on the "miraculous." But large amounts of the drug, as well as of streptomycin and PAS, became available on the open market—and in Korea one needs no prescription to buy any drug.

In 1955 we began to note a lessened response to the antituberculous drugs, despite increased dosage of isoniazid. Seeing an average of 100 cases of tuberculous meningitis yearly in one pediatric clinic and the proportionately much larger number of pulmonary cases, one gets "hunches" even without statistical study. Slowly we began to realize that what had been a blessing was becoming a curse. Patients who formerly had a rapid downhill course, confining them at least to bed, now bought medicine as they pleased, gained weight, and returned to work. Most of them could not afford the needed rest until sputum was negative, and felt well enough to work while expectorating their tubercle bacilli in ever-widening circles. True, the disease usually catches up with them as they become drug resistant, and the end is the same as in pre-drug days. But, meantime, consider the number of people exposed to just one such patient. Where is all this leading? Quite surely right back to the days before antituberculous drugs were available, and I think in Korea that future is not far away.

This, of course, is not likely to happen in America where drugs are given only under medical supervision. However, the tendency to home care of even sputum-positive patients to me seems inadvisable. Here in Korea institution care is so extremely limited as to be, from a practical point of view, unavailable. From the enormity of the problem it seems of first concern, at least, to confine patients to their homes so that contacts will be only in the immediate family, which can be kept under close surveillance. But this is not an ideal program, and surely America should strive for the ideal.

The great danger of home care was so strikingly brought home to me today as to finally move me to write this letter so long contemplated. About three weeks ago a five-year-old child was brought to us with tuberculous meningitis. The home situation revealed the child's father to have active pulmonary tuberculosis, treated for five years with all three drugs. He appears well, is ambulatory, and one finds it hard to believe the chest findings of far advanced bilateral disease with multiple cavities. The child's mother also has tuberculosis, much treated for three years, and the disease is apparently arrested at present.

The child is being treated with cortisone, streptomycin, and isoniazid (30 mg. per kg.) and is still spastic and comatose. Today, the only other child, two years old, was brought

to the clinic with a history of vomiting for five days, drowsiness for three days, and spinal fluid typical of tuberculous meningitis also. To my mind, this family history is a stronger plea than any I could make to treat cases of active pulmonary tuberculosis in an institution if it is at all possible.

May I take this opportunity, please, to thank you and your editorial board for the fine material always presented in your Review? We like to know the best even if circumstances sometimes prevent our doing it.

Sincerely yours,

MARYKNOLL SISTERS
P. O. BOX 77
PUSAN, KOREA

SISTER AGNES THERESE SIMPLER

April 10, 1957

CONSIDERATIONS OF FALSE POSITIVE REACTIONS IN THE GLOBULIN TITRATION TECHNIQUE AS APPLIED TO TUBERCULOSIS[1]

To the Editor of The American Review of Tuberculosis and Pulmonary Diseases:

We have recently reported the demonstration of a circulating antibody in patients with active tuberculosis by a new concept in serologic testing.[2] In the report we noted three false positive tests among 74 normal persons tested. Each of these patients had suffered an upper respiratory infection of an undefined nature within the preceding few weeks. Tests for cross reactivity with antibodies to Influenza *B*, Coxackie *A*, and *APC* virus proved negative.

We have, since the last report, determined that in 2 patients with *Beta* hemolytic streptococcal infections (*Group A*) and one patient with *Beta* hemolytic streptococcus (*Group D*), positive serologic titers for tuberculosis antibody were demonstrated. These 3 patients also demonstrated elevated Antistreptolysin® "O" titers (ASO). Subsequently, in a study of the presence of Antistreptolysin "O" antibodies in the sera of 30 patients with active tuberculosis, 28 demonstrated ASO titers of 100–500 Todd Units (TU). The mean was 210 TU. Two patients repeatedly tested showed negative ASO titers but positive tuberculosis serology.

In addition, we have made the following observations which should be considered in evaluating the results of the "globulin titration serologic test for tuberculosis."

1. Rabbit anti-human serum (Ortho Lot R7087-2)[3] did not contain either Antistreptolysin "O" antibodies or tuberculosis antibodies.

2. Some sera lose measurable tuberculosis antibody in 21 days at 4°C. instead of at 45 days as previously reported.

3. Serum which has been frozen loses demonstrable tuberculosis antibody.

4. When sera from 18 patients with active tuberculosis were tested with antigen which had been frozen and thawed, 16 failed to demonstrate antibodies. The remaining two were discovered to be patients with a history of chronic tuberculosis for at least 5 years. Further studies on the antigenic nature of the lysates and antibodies measured are being pursued in relation to this finding.

[1] This investigation was supported in part by a research grant (E 1393) from the Institute of Allergy and Infectious Diseases, National Institutes of Health, Public Health Service.

[2] FUSILLO, M. H., *et al.*: Am. Rev. Tuberc., 1957, *75*, 954.

[3] Kindly supplied by Ortho Pharmaceutical Co., Raritan, N. J.

5. Sera from 4 additional hospitalized patients with disease clinically compatible with sarcoidosis demonstrated antibodies to tuberculosis by the technique. The two previous nonhospitalized patients had negative titers.

6. Positive ASO sera were adsorbed with tuberculosis antigen and were retested after adsorption. ASO antibody was adsorbed and resulting ASO tests were negative. We are currently attempting to adsorb positive tuberculosis sera with certain antigenic fractions of *Beta* hemolytic streptococci.

Inasmuch as formalin is recognized as an excellent substance for preserving the antigenic components of bacteria, by the method of assay used, we believe we are measuring antibodies to surface antigens of *M. tuberculosis*. Raffel[4] has described a lipoid factor in *Group C* hemolytic streptococci that is said to be similar to the wax substance of tubercle bacilli. Ehrenkranz and Waksman[5] have recently demonstrated what appears to be active induction of tuberculin hypersensitivity in guinea pigs infected with *Group C* streptococci. Halpern and colleagues[6] have demonstrated a cross-reacting antibody in *Group C* streptococci by the use of fluorescent antibody.

Studies are planned to determine if a relationship exists between surface antigens of *M. tuberculosis*, Streptococci, Staphylococci, Pneumococci, and the toxin of *Clostridium welchii*.

Sections for Infectious Diseases
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Matthew H. Fusillo
Daniel L. Weiss

George Washington University
School of Medicine
Washington, D. C.

April 25, 1957

[4] Raffel, S.: In *Progress in Allergy*, edited by P. Kallos, Little Brown Co., Boston, 1954, p. 173.
[5] Ehrenkranz, N. J., and Waksman, B. H.: J. Exper. Med., 1956, *104*, 935.
[6] Halpern, S., *et al.*: Texas Rep. Biol. & Med., 1957, *15*, 63-64.

Surgical vs. Nonsurgical Treatment of "Open-Negative" Syndrome

To the Editor of The American Review of Tuberculosis and Pulmonary Diseases:

Bell and associates[1] have recently refocused attention on the advisability of the surgical removal of the pulmonary lesions from tuberculous patients with *the "open-negative" syndrome*. By their criteria these are persons with planigraphic evidence of annular shadows and sputum cultures repeatedly negative for *M. tuberculosis* for three months or more by the eighth month of their initial course of antituberculous chemotherapy. To judge from their data on the morphology and bacteriology of the lesions resected from 153 such patients, only a small proportion of these patients revealed complete so-called "open-healing."[2–5] A very thorough one- to five-year follow-up after the eighth month observation point revealed an *8.4 per cent relapse rate* in these surgically treated patients.

[1] Bell, W., *et al.*: Am. Rev. Tuberc., 1957, *75*, 538.
[2] Johnsen, L., and Hewitt, W. C.: Am. Rev. Tuberc., 1954, *69*, 1054.
[3] Thompson, J. R.: Am. Rev. Tuberc., 1955, *72*, 601.
[4] Bernou, A., and Brun, J.: Presse méd., 1955, *63*, 894.
[5] Corpe, R. F., and Stergus, I.: Am. Rev. Tuberc., 1957, *74*, 223.

There were 118 patients with the same syndrome who did not have resection for various reasons, including various medical and surgical contraindications, refusal of operation, *elective thoracoplasty*, and elective nonsurgical therapy. A one- to five-year follow-up of this group revealed a *43 per cent relapse rate* with a tendency for relapse to be more severe in the latter nonresected group.

The writer would like to take issue with Bell and associates on two points:

1. The implication that elective thoracoplasty is much less effective than resection in these cases is made, and if true, should be more carefully documented in the light of excellent past experience with this surgical collapse method.
2. They have lumped together the various antituberculous regimens initiated before December, 1954, as if there was no good evidence of real differences in their relative effectiveness at this time. Since isoniazid was not introduced until early in 1952, only about 25 per cent of these patients did in fact receive initial treatment including isoniazid.[6] If the patients had been divided into those receiving (*1*) streptomycin 1 gm. twice-weekly-para-aminosalicylic acid, (*2*) streptomycin 1 gm./day-para-aminosalicylic acid, (*3*) streptomycin 1 gm. twice weekly-isoniazid 300 mg./day and, (*4*) isoniazid 300 mg./day-para-aminosalicylic acid, there is good reason to believe that the isoniazid-para-aminosalicylic acid patients would at least have fared better than the streptomycin 1 gm. twice-weekly para-aminosalicylic acid patients at comparable points of observation.[4]

It appears that relapse is quite apt to occur during the first twelve months after the open-negative state has been achieved at the sixth to eighth month of predominantly streptomycin-para-aminosalicylic acid chemotherapy, provided the open lesions are not removed. Patients who attain the open-negative status after six to eight months of primary isoniazid-para-aminosalicylic acid therapy, on the other hand, are *not* apt to relapse during the ensuing twelve months of continued isoniazid-para-aminosalicylic acid therapy; this is not only the writer's experience, but also that of others.[7–9]

Colorado Foundation for Research in Tuberculosis
4200 East Ninth Avenue
Denver, Colorado

Roger S. Mitchell

May 24, 1957

[6] Raleigh, J. W.: Personal communication.
[7] Corpe, R. F., *et al.*: The fate of the open-negative patient. Unpublished data presented at annual meeting of the American Trudeau Society, May, 1957.
[8] Johnsen, L.: Personal communication.
[9] Corpe, R. L.: Personal communication.

Books

ERGEBNISSE DER GESAMTEN TUBERKULOSE-FORSCHUNG. vol. XIII. Edited by St. Engel, L. Heilmeyer, J. Hein, and E. Uehlinger, pp. 659, George Thieme Verlag, Stuttgart, 1956. Price, $22.85.

This is the thirteenth volume of a series of publications which appear in irregular sequence to cover the field of tuberculosis and respiratory diseases both from the scientific and the clinical standpoint. The present volume is devoted entirely to problems related to various aspects of the antimicrobial treatment of tuberculosis. Special attention is directed to the problems of resistance, and the indications fo antimicrobial surgical treatment are discussed.

An introductory article by Dr. Koenn discusses the histologic changes which occur during various manifestations of tuberculosis under antimicrobial medication. Whichever drug is used, the histologic picture of the healing lesion is the same and does not differ from that seen in spontaneous healing.

The chapter by Dr. Heilmeyer following the introductory article presents a thorough discussion of the problem of resistance together with an elaborate review of both the Continental and American literature. Starting with Ehrlich's early observations of resistance in infections with different strains of Trypanosoma, the author gives a concept of the basic laws and biochemical mechanisms of resistance. Numerous tables relate the experiences gained during administration of single drugs and combined drug regimens. Isoniazid-streptomycin appears to be the method of choice.

The next article, by Dr. Schaich, deals with the problems of untoward reactions observed during antimicrobial treatment: impairment of vestibular and cochlear function, eosinophilia, hematuria, albuminuria, liver damage, contact dermatitis in streptomycin treatment; neuritis, damage to the central nervous system; cardiovascular disturbances, decrease of capillary resistance, Cushing-like pictures under isoniazid treatment; allergic manifestations, gastrointestinal disturbances, cardiac arrhythmias, rarely agranulocytosis under PAS treatment; and the severe depression of leukocyte formation not infrequently observed under treatment with thiosemicarbazone. Neomycin, viomycin, and mycomycin, which are rarely used in Europe, are briefly mentioned.

Dr. Bruegger's contribution reviews his experiences in the treatment of children. He noted good results in pulmonary and genitourinary tuberculosis and less satisfactory results in tuberculosis of the skeleton. Dr. Gloor's article on genitourinary tuberculosis emphasizes the importance of hematogeneous infection in renal tuberculosis. Intensive treatment with antimicrobials is advised before surgical approach be considered.

Doctors Kirchhoff and Strauebig review their results in the therapy of uterine and ovarian tuberculosis. In their opinion, the antimicrobials have brought about a decisive turn for the better in previously unsatisfactory results.

Dr. Schaich discusses the extremely important topic of tuberculosis in pregnancy. The antimicrobial era has also brought to the field an important change. Artificial abortion is indicated only when genital tuberculosis or eye tuberculosis are present.

The entire scale of varying and partly contradictory opinions in the world literature regarding the treatment of skeletal tuberculosis are reflected in Dr. Ullmann's article on this subject. The antimicrobial drugs have shortened considerably the period of complete immobilization and made the surgical procedures much more rewarding.

Dr. Zintz, in his contribution on the problems of eye tuberculosis, comes to the same

conclusions as the majority of investigators: All manifestations of this disease located *in* the eye respond rather poorly to antimicrobial treatment, and the classical methods of therapy have maintained their value.

Dr. Link sees definitely improved results in tuberculosis of the middle ear under treatment with various antimicrobial regimens.

Dr. Loeffler and Dr. Jaccard review the world literature and their own results in the treatment of tuberculous pericarditis and peritonitis (very satisfactory), polyserositis (less significant), and pleuritis (no result whatsoever).

The problem of tuberculosis of the skin is discussed by Dr. Kalkhoff. Lupus vulgaris responds very well to isoniazid-vitamin D_2 treatment combined with Kromayer irradiation. The non-lupus forms of tuberculosis of the skin also give good results with antimicrobial treatment alone or in combination with vitamin D_2 except the lichen scrofulosorum and the papulonecrotic tuberculid, in which the addition of vitamin D_2 seems to be of no particular avail.

Dr. Kleinschmidt advises immediate treatment with a drug combination (isoniazid-streptomycin) in all cases of tuberculous meningitis. A rapid response is observed in a great number of cases, and the relapse rate is quite low. BCG vaccination has proved its value as a prophylactic measure.

Drs. Schaefer and Witte stress the importance of isoniazid-streptomycin in the treatment of infants, not so much because of its influence upon the primary complex but mainly as a dependable preventive measure against hematogeneous spread. Dr. Bruegger believes that surgery is still the method of choice in treating tuberculous peripheral lymph nodes, particularly in the neck. Histologic studies have revealed that a large number of nodes not showing disease macroscopically do show definite changes when subjected to histologic examination. Under antimicrobial cover, surgery is a safe procedure, while X-ray is of no avail.

Dr. Arold has found that thiosemicarbazone, vitamin D_2, and isoniazid are superior to any other drug in the treatment of tuberculosis of the upper respiratory tract.

In Dr. Berg's article on gastrointestinal tuberculosis, satisfactory results have been obtained with practically all available drugs. Thiosemicarbazone seems to be the drug of choice because of its penetrating effects upon the mucous membranes. Surgery will usually be unnecessary unless stenosis or perforation of the intestines occur.

Doctors Hein and Stecher review the classical topic of the treatment of pulmonary tuberculosis. While isoniazid, streptomycin, and PAS are usually employed, viomycin and cycloserine should be kept in reserve. A somewhat critical attitude is maintained as to whether the value of drug combinations is really based upon delayed development of resistance. The question of long-term treatment is considered to be unsolved at the present time.

Doctors Nissen and Forschbach discuss in detail advantages and disadvantages of the surgical approach. The intrapleural and extrapleural pneumothorax still can be of considerable value in well-selected cases (small cavities of the apices in absence of bronchial tuberculosis). The extrapleural plombage is merely an emergency measure in cavity hemorrhages which do not respond to any other form of treatment and in old age, when neither resection nor pneumothorax are advisable. Thoracoplasty is reserved for cases of incomplete expansion of the operated lung after resection of one lobe, tuberculous empyema, and bilateral lesions not considered to be a good risk for resection. The direct treatment of cavities is antiquated now that resection is giving satisfactory and definite results. Resection should be done in persistent cavities, destroyed lung, or bronchial stenosis. The patient should have overcome the exudative phase of the disease at the

time of surgery. Large tuberculomas should be resected under all circumstances. The main complications, bronchopleural fistula and empyema, are still serious incidents. The value of pre- and postoperative sanatorium therapy is emphasized. Decortication has become more rewarding under antimicrobial cover.

The high level of the contributions, extensive reviews of the literature in every field, excellent illustrations, and clear tables make this book a valuable source of information for every investigator engaged in the field of tuberculosis.

DIETHELM BÖHME
New York, New York

ROENTGEN SIGNS IN CLINICAL DIAGNOSIS. By Isadore Meschan. pp. 1058, W. B. Saunders Company, Philadelphia and London, 1956. Price, $20.00.

This volume, approximately 1,000 pages in length, well written and well illustrated, covers the broad field of diagnostic radiology with a thoroughness rarely attempted in single-volume textbooks. Unfortunately, the inevitable production costs have placed it in the "expensive" bracket and, as a consequence, it may not find widespread application as a textbook for medical students.

The author has made an attempt to present "the fundamentals of radiology rearranged on the basis of objective signs as seen in roentgenograms." Understandably, he has not been completely successful. Any effort to classify so broad a subject as diagnostic radiology would necessarily be somewhat artificial. However, the author's approach seems justifiable on purely pragmatic grounds.

The illustrations are unusually good. Particularly commendable are the pen and ink drawings of radiographs, many of which succeed in achieving a three-dimensional appearance. This technique is at its best in the drawings which are used liberally in the chapters devoted to the radiology of the chest.

Despite its length, the volume necessarily is the product of considerable selectivity upon the part of its author. It is believed that the selection has been competent. It is possible that in a subsequent edition the author may wish to expand Chapter Two, which deals with protection from roentgen irradiation.

CHARLES T. DOTTER
Portland, Oregon

BOOKS RECEIVED

THE INFECTIOUS DISEASES OF DOMESTIC ANIMALS. With Special Reference to Etiology, Diagnosis, and Biologic Therapy. Third Edition. By William Arthur Hagan and Dorsey William Bruner. Pp. 968, Cornell University Press, Ithaca, New York, 1957. Price, $10.50.

ANNUAL REVIEW OF MEDICINE. Vol. 8. By David A. Rytand and William Creger. Pp. 530, Annual Reviews, Inc., Palo Alto, California, 1957. Price, $7.00.

BCG VACCINATION AGAINST TUBERCULOSIS. By Sol Roy Rosenthal. Pp. 389, Little, Brown & Company, Boston, Massachusetts, 1957. Price, $7.50.

THE CHEMISTRY AND BIOLOGY OF PURINES. Ciba Foundation Symposium. Edited by G. E. W. Wolstenholme and Cecilia M. O'Connor. Pp. 327, Little, Brown and Company, Boston, Massachusetts, 1957. Price, $9.00.

REGULATION AND MODE OF ACTION OF THYROID HORMONES. Ciba Foundation Symposium. Edited by G. E. W. Wolstenholme and Elaine C. P. Millar. Pp. 311, Little, Brown and Company, Boston, Massachusetts, 1957. Price, $8.50.

American Trudeau Society

Medical Section of the National Tuberculosis Association

NOTICES

The following dates and locations of the next two annual meetings of the National Tuberculosis Association and the American Trudeau Society have been selected and approved:

1958—Philadelphia, Pennsylvania—May 19–24
1959—Chicago, Illinois—May 24–29

ANNUAL MEETING OF THE AMERICAN TRUDEAU SOCIETY

The Medical Sessions Committee INVITES SUBMISSION OF PAPERS on all scientific aspects of tuberculosis and nontuberculous respiratory and cardiopulmonary diseases for presentation at the *1958 annual meeting in Philadelphia, Pennsylvania, May 19–21.*

Abstracts

1. Abstracts of papers to be offered for presentation should be submitted before January 10, 1958, to the Chairman of the Medical Sessions Committee. *Five copies* are required for distribution to committee members.
2. Abstracts should be limited to a 300-word summary including important data *and conclusions.* Charts and tables should be attached to aid in the selection of papers.
3. The names, academic degrees, titles, and complete addresses of the senior author and co-authors should be included in each abstract. The author who is to present the paper should be the first author listed in the abstract.
4. All abstracts will be reviewed by the Medical Sessions Committee. Selection will be largely based on the areas of interest suggested by you and others receiving this invitation.
5. Those wishing to present subjects of a confidential nature from the armed forces or other sources should first clear the material through official channels.
6. Authors will be notified by March 1, 1958, if their papers have been accepted for presentation.
7. Papers presented at the annual meeting are expected to be original contributions not previously presented or published. Medical papers presented at the meeting may be submitted to THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES by the author if he so desires.

Suggestions

The Medical Sessions Committee is particularly anxious to build the 1958 program around your special interests. A memorandum suggesting subjects and speakers would be welcome and should be sent to the Chairman by October 15, 1957.

KATHARINE R. BOUCOT, M.D., *Chairman*
Medical Sessions Committee
Woman's Medical College
Philadelphia 29, Pennsylvania

EXHIBITS FOR 1958 NTA-ATS ANNUAL MEETING, MAY 19 TO MAY 22, PHILADELPHIA, PA.

NTA and ATS Members who have ideas or materials for Exhibits, Movies, or TV Programs please write to Dr. E. Wayne Marshall, *Chairman*, 1958 Exhibits Subcommittee, 1520 Spruce Street, Philadelphia 2, Pa.

NEWLY ELECTED OFFICERS OF STATE TRUDEAU SECTIONS

Arizona

Pres. James C. Soderstrom, M.D.
Vice-Pres. Richard O. Flynn, M.D.
Secy-Treas. Harold E. Kosanke, M.D.

Arkansas

Pres. Harley C. Darnall, M.D.
Pres-Elect Andrew Pringos, M.D.
Secy-Treas. Dorothy Cody, M.D.

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Vice-Pres. Ralph E. Smiley, M.D.
Secy-Treas. Paul M. Seebohm, M.D.

Kansas

Pres. Martin J. FitzPatrick, M.D. (until 9/57)
Pres. Clarence W. Erickson, M.D. (effective 9/15/57)
Pres-Elect Roy A. Lawson, Jr., M.D. (1958)
Secy-Treas. John K. Fulton, M.D.

Kentucky

Pres. Jack Chumley, M.D.
Vice-Pres. Oren A. Beatty, M.D.
Secy-Treas. Boyce E. Jones, M.D.

Louisiana

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Pres-Elect Peachy R. Gilmer, M.D.
Secy-Treas. Joseph K. Bradford, M.D.

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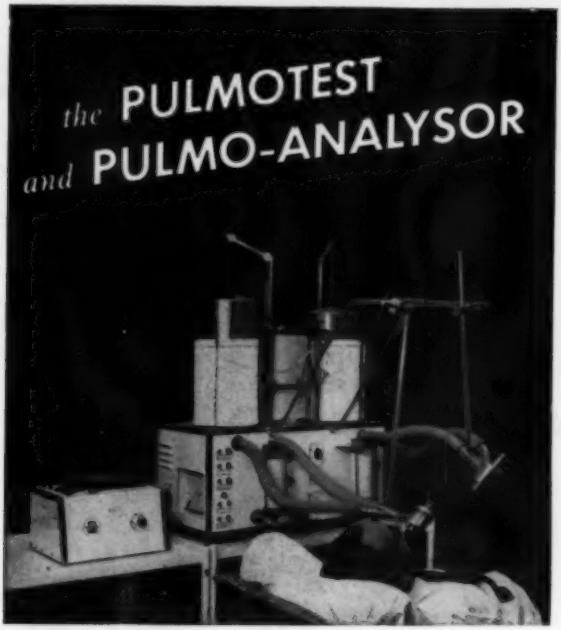

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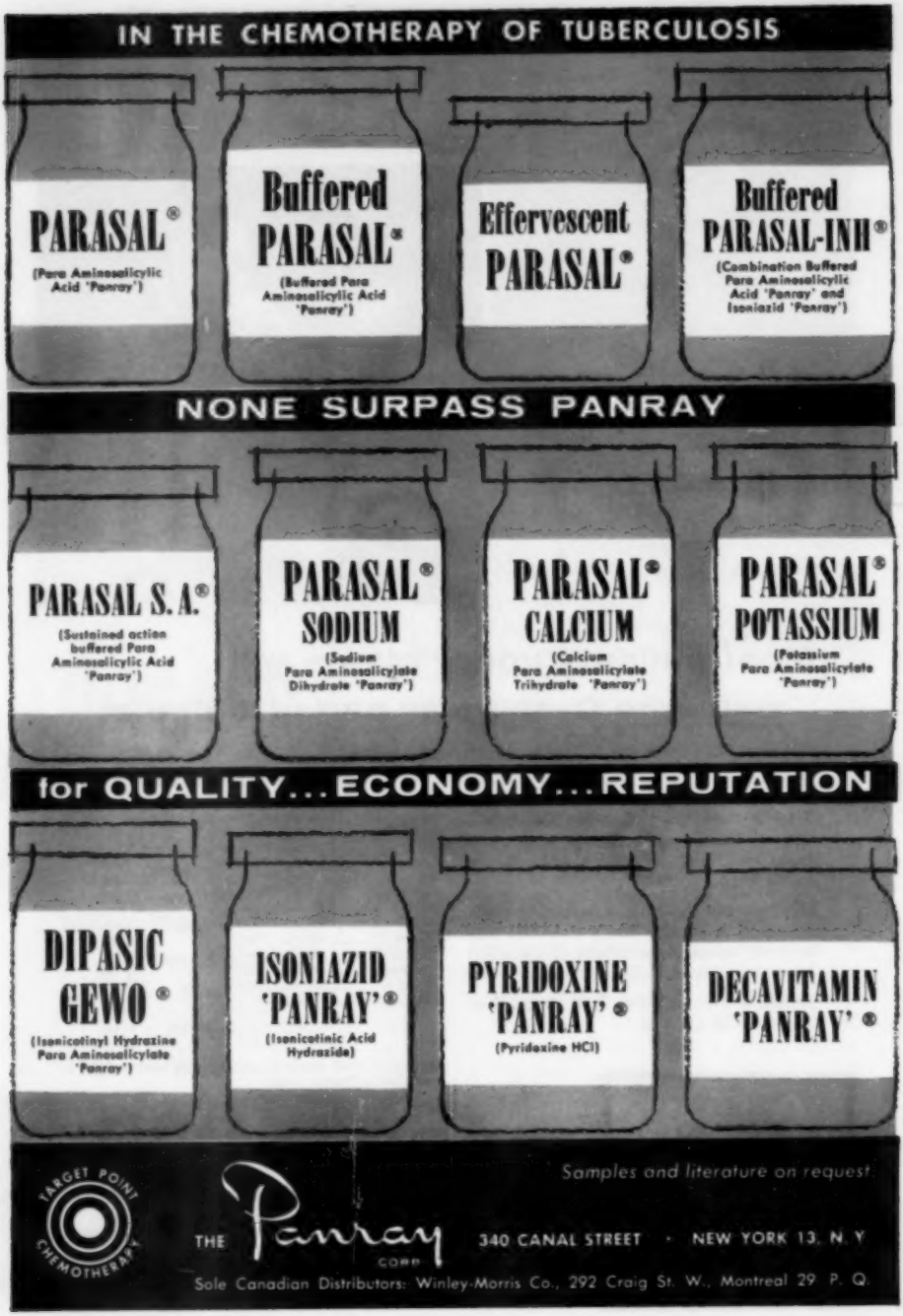
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